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(57) Abstract

The present invention provides a purified bacterial protein expressed during infection due to streptococci or enterococci and isolated from human sera, together with immunogenic fragments, analogs, inhibitors, antibodies and antigenic fragments specific thereto. Also provided is a DNA sequence coding for a bacterial protein or an immunogenic fragment or an analogue thereof expressed during infection due to Streptococci or Enterococci, together with homologues thereof, together with vectors, probes and inhibitors therefor. Also provided is fibronectin or an immunogenic fragment thereof or an analogue thereof or an antibody thereto or an antigen binding fragment thereof when used in a method of treatment or diagnosis of the human or animal body for infection due to Streptococci or Enterococci. Also provided are antibodies specific to HSP 90 or immunogenic fragments or analogues thereof for use in a method of diagnosis or treatment of the human or animal body of infection due to streptococci or enterococci due to any one of the group of *S.oralis*, *S.gordonii*, *S.sanguis*.

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Diagnosis and Treatment of Infections due to Streptococci and Enterococci

This invention concerns the diagnosis, prophylaxis and treatment of infections due to Streptococci and Enterococci, especially endocarditis and septicaemia.

Endocarditis is commonly caused by Streptococcal and Enterococcal infection. These are bacterial species which grow in the heart valves of an infected patient and cause damage thereto. Endocarditis is currently diagnosed by clinical features, echocardiogram and the presence of heart murmurs. The causative microorganism is usually identified by blood culture (culture-positive endocarditis). However, in approximately 10% of infective endocarditis patients the blood culture is negative. This may lead to a wrong diagnosis and/or delayed treatment.

Active infective endocarditis in which blood cultures are negative has been a recognised clinical entity since the beginning of the century. Etiological factors involved in such culture-negative endocarditis include (1) previous antibiotic therapy, (2) fastidious, slow growing bacteria and (3) non-bacterial organisms.

Patients with rheumatic fever, damaged heart valves or prosthetic valves are at risk of a secondary Streptococcal infection leading to endocarditis when having routine dental or gastrointestinal procedures.

Additionally, a growing problem in recent years has been the spread of

vancomycin-resistnt enterococci (VRE). The emergence of enterococci resistant to most or all licensed antibiotics leaves few treatment options and recent studies have shown that 36.6% of those patients with VRE in blood died as compared with 16.4% of those with vancomycin sensitive enterococci. Clearly a treatment for enterococci of all descriptions, and especially of VRE is extremely desirable.

One method of diagnosing endocarditis and, more particularly, culture-negative endocarditis, is by immunoblotting sera of such patients to reveal raised levels of antibodies against causative microorganisms and also a pattern on immunoblot which appears to be species specific (Clark & Burnie, J.Clin.Pathol. 1991, 44, 152-156; Burnie *et al.*, J. Clin. Pathol, 1987, 40: 1149-1158; Burnie & Clark, J. Immunol. Methods, 1989, 123: 217-225). However, although individual species of both Streptococcus and Enterococcus can be identified, there is some cross- reaction between species making absolute diagnosis sometimes difficult. Immunoblotting is also cumbersome as well as expensive.

Current therapy of both culture-positive and culture-negative endocarditis involves antibiotics, however, some of the antibiotics necessary to treat endocarditis are highly toxic, for example, vancomycin and gentamicin may be nephrotoxic and ototoxic. Additionally, it is difficult to assess patient response to antibiotic treatment since, although the organism may no longer be viable, fever may persist.

The present invention provides a method of diagnosis and treatment ("treatment" from herein being taken to include prophylaxis) of infections due to Streptocci and Enterococci which is specific to both culture-positive and culture-negative

endocarditis, thereby overcoming at least to some extent, the aforesaid problems.

Experiments were undertaken which resulted in the cloning, sequencing and characterisation of Streptococcal antigens, resulting in the determination of an antigen expressed during infection due to Streptococci and Enterococci, together with immunogenic fragments thereof which were found to be useful in the prophylaxis and treatment of infections due to both Streptococci and Enterococci.

According to the present invention there is provided a purified bacterial protein expressed during infection due to streptococci or enterococci and isolated from human sera having at least the sequence of formula (1):

```
NH-      10                  30                  50
EFTFYDENDQ PINFDNALLS VASLNREHNS IEMAKDYSGT FIKISGSSIG EKNGMIYATE
         70                  90                  110
TLNFKQGQGG ARWTMYPNRQ PGSGWDSSDA PNSWYGAGAI SMSGPTNHVT VGATSATNVM
        130                 150                 170
SVAEMPQVPG RDNTEGKRPN IWYSLNGKIR AVDVPKITKE KPTPPVAPTE PQAPTYEVEK
        190                 210                 230
PLEPAPVAPS YENEPTPPVK TPDQPEPSKP EEPTYETEKP LEPAPVAPNY ENEPTPPVKT
        250                 270                 290
PDQPDPSKPE EPNYETEKPL EPAPVAPSYE NEPTPPVKTP DQPEPSKPEE PNYDPLPTPP
        310                 330                 350
LAPTPKQLPT PPAVPTVHFH YNRLFAQPQI NKEIKNEDGV DIDRTLVAKQ SVVKFELKTE
        370                 390                 410
ALTAGRPKTT SFVLVDPLPT GYQFDLEATK AASKGFETSY DKASHTVTFK ATEETLAAFN
```

```
       430                   450                   470
ADLTKSFETL YPTVVGRVLN DGATYTNNFT LTVNDATGVK SNIVRVTTPG KPNDPDNPNN
       490                   510                   530
NYIKPLKVNK NKQGVNIDGK EVLAGSTNYY ELTWDLDQYK GDKSSKEAIQ NGFYYVDDYP
       550                   570                   590
EEALTLQPEL VKIRDLEGNL VSGISVQQFD SLERAPKKVQ DLLKKANITV KGAFQLFSAD
       610
NPAEF
```

or an immunogenic fragment thereof or an analogue thereof.

The single letters in formula (1) are each to be understood to represent a separate amino acid, and each is the conventional single letter symbol used for amino acids.

Bacterial protiens expressed during infection due to Streptococci or Enterococci according to the invention include those proteins wherein one or more amino acids in the sequence of formula (1) is replaced by another amino acid, providing that overall functionality of the protein is conserved.

A bacterial protein according to the invention may be further characterised by either one or both of the following features:-

(1) It is an immunodominant conserved antigen; and

(2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection;

A bacterial protein according to the invention may also be characterised in that it is involved in binding to heart valves.

The bacterial protein may be obtained from one of the group of Streptococcus oralis, Streptococcus sobrinus, Streptococcus gordonii, Streptoccocus sanguis, Streptococcus mutans, Streptococcus mitis, Streptococcus mitior, Streptococcus parasanguis, Streptococcus bovis, Enterococcus faecalis and Enterococcus faecium.

Additionally, the bacterial protein may be obtained from either one of the group of vancomycin-resistant Enterococcus faecalis and Enterococcus faecium.

Particular fragments of a bacterial protein expressed during infection due to Streptococci or Enterococci include any peptide epitopes ("immunogenic fragments"), for example, a few amino acids or analogues thereof. Examples of such epitopes include YEVEKPLEPAPVAPS, SYENEPTPPVKTPD, KTPDQPEPSKPEEPT, EPAPVAPSYENEPTP, YEVEKELVDLPVEPS, KTPDQNIPDKPVEPT, TMYPNRQPGSGWDSS and WYSLNGKIRAVDVPK. Peptides of this type may be synthesised using conventional liquid or solid phase peptide synthesis techniques.

In a further aspect the invention particularly provides a recombinant bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof, or an analogue thereof.

As mentioned earlier, current treatment of endocarditis is by antibiotics and recovery is often difficult to assess as fever may persist after other symptoms have been relieved. Tests currently available to measure the efficiency of antibiotic treatment, for example, minimum inhibitory concentration, minimum bactericidal concentrations and back titrations, measure only organism sensitivity not actual organism death in the patient. We show for the first time, as detailed below, that in patients with endocarditis due to Streptococcus oralis, S. gordonii, S. sanguis and S. mitis and undergoing antibiotic therapy, resolution of the disease was accompanied by at least a 50% drop in IgM titre within two weeks. This provides a direct marker of successful antibiotic therapy showing for the first time a direct marker of pathogen kill. Since the IgM antibody is specific to endocarditis it can be used in the diagnosis of both culture-positive and, more particularly, culture-negative endocarditis.

Thus the present invention also provides a method of diagnosis of culture-positive and culture-negative endocarditis using IgM antibody to a bacterial protein expressed during infection due to Streptococci or Enterococci, said bacterial protein, and therefore IgM antibody raised thereto, falling by 50% within two weeks after commencement of antibiotic treatment, thereby acting as a marker of pathogen kill.

In another use the bacterial protein according to the present invention may be employed, using conventional techniques, for screening to obtain activity inhibiting agents for use in the prophylaxis and treatment due to Streptococci or Enterococci and in particular of culture-positive and culture-negative endocarditis-causing bacterial infection. Such screening methods forms a further aspect of the invention.

In a further use, the bacterial protein according to the invention is particularly well suited for the generation of antibodies. Thus according to a further aspect of the invention we provide a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof or an analogue thereof, for use as an immunogen.

Standard immunological techniques may be employed with the bacterial protein in order to use it as an immunogen. Thus, for example, any suitable host may be injected with the protein and the serum collected to yield the desired polyclonal anti-bacterial protein antibody after purification and/or concentration. Prior to injection of the host the bacterial protein may be formulated in a suitable vehicle and thus according to a further aspect of the invention we provide a composition comprising a bacterial protein expressed during infection due to Streptococci or Enterococci and having an amino acid sequence which includes at least the sequence of formula (1) or an analogue thereof together with a pharmaceutically acceptable carrier, diluent or excipient.

For purification of any anti-bacterial protein antibody, use may be made of affinity chromatography employing an immobilised bacterial protein of the present invention as the affinity medium. Thus according to another aspect of the invention we provide a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1), or an immunogenic fragment thereof or an analogue thereof, covalently bound to an insoluble support.

Various derivatives of the bacterial protein or fragment or analogue may be used to inhibit said protein, fragment or analogue. The use of the bacterial protein expressed during infection according to the invention as immunogens for the production of antibodies generates one type of inhibitor of the action of the protein. Generally, inhibitors of the bacterial protein are potentially useful in the diagnosis, and in particular the prevention and treatment, of infections due to Streptococci or Enterococci and in particular of both culture-positive and culture-negative endocarditis, and provide a further feature of the invention. Inhibitors include any antagonists of the action of the bacterial protein expressed during infection or agents which prevent their production, and in particular those which may be used in treatment of endocarditis-causing bacterial infections. Suitable inhibitors include, for example, pharmaceutical reagents, including antibodies, and chemical analogues of the bacterial protein expressed during infection to antagonise the action of the bacterial protein, and anti-sense RNA and DNA to prevent production of the bacterial protein. Suitable inhibitors may be determined using appropriate screens, for example, by measuring the ability of a potential inhibitor to antagonise the action of, or prevent the production of a bacterial protein expressed during infection due to Streptococci or Enterococci according to the invention or an immunogenic fragment thereof, or an analogue thereof, in a test model for example an animal model such as the mouse model.

It will also be appreciated that by suitable epitope mapping using conventional procedures[Geysen *et al.*, J. Immunol. Methods, 102: 259-274 (1987); Hopp and Woods, PNAS USA, 78(6): 3824-3828 (1981); Novotny *et al.*, PNAS USA, 83: 226-230 (1986)], peptide fragments of the bacterial protein expressed during infection may be identified which can be chemically synthesised. Synthetic peptide

antigens of this type may be used to produce inhibitors e.g. to raise antibodies for use in diagnosis and/or therapy, as previously described, or to produce antiseras, e.g. non-specific polyclonal antisera, for use as a vaccine, and as discussed above form a further aspect of the invention.

According to a further aspect of the invention we provide a derivative of a bacterial protein expressed during infection due to Streptococci or Enterococci, said protein having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof or an analogue thereof, wherein the derivative inhibits said protein, fragment or analogue.

Such inhibitors may be used either alone or where appropriate in combination with other pharmaceutical agents, for example, other antibiotics.

One particularly useful group of inhibitors according to this aspect of the invention are antibodies capable of recognising and binding to the bacterial proteins.

Thus according to yet another aspect of the invention we provide isolated and purified antibody specific for one or more epitopes of a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof or an analogue thereof.

The antibody may be a whole antibody or an antigen binding fragment thereof and may in general belong to any immunoglobulin class. Thus, for example, it

may be an immunoglobulin M antibody or, in particular, an immunoglobulin G antibody. The antibody or fragment may be of animal, for example, mammalian origin and may be for example of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or, if desired, a recombinant antibody fragment, ie., an antibody or antibody fragment which has been produced using recombinant DNA techniques.

Particular recombinant antibodies or antibody fragments include human recombinant antibodies and in particular include (1) those having an antigen binding site at least part of which is derived from a different antibody, for example those in which the hypervariable or complementarity determining regions of one antibody have been grafted into the variable framework regions of a second, different antibody (as described in European Patent Specification No 239400); (2) recombinant antibodies or fragments wherein non-Fv sequences have been substituted by non-Fv sequences from other, different antibodies (as described in European Patent Specification Nos 171469, 173494 and 194276); or (3) recombinant antibodies or fragments possessing substantially the structure of a natural immunoglobulin but wherein the hinge region has a different number of cysteine residues from that found in the natural immunoglobulin but wherein one or more cysteine residues in a surface pocket of the recombinant antibody or fragment is in the place of another amino acid residue present in the natural immunoglobulin (as described in International Patent Application Nos PCT/GB88/00730 and PCT/GB88/00729).

The antibody or antibody fragment may be of polyclonal, or preferably, monoclonal origin. It may be specific for a single epitope or for a number of epitopes associated with the bacterial protein.

Antigen binding antibody fragments include, for example, fragments derived by proteolytic cleavage of a whole antibody, such as F(ab')2,Fab' or Fab fragments, or fragments obtained by recombinant DNA techniques, for example Fv fragments (as described in International Patent Application No PCT/GB88/0747).

The antibodies according to the invention may be prepared using well-known immunological techniques employing the bacterial protein expressed during infection as antigen. Thus, for example, any suitable host may be injected with the bacterial protein and the serum collected to yield the desired polyclonal antibody after appropriate purification and/or concentration (for example by affinity chromatography using the immobilised bacterial protein as the affinity medium). Alternatively, splenocytes or lymphocytes may be recovered from the bacterial protein-injected host and immortalised using for example the method of Kohler *et al.*, Eur. J. Immunol. 6: 511, 1976, the resulting cells being segregated to obtain a single genetic line producing monoclonal anti-streptococcal or -enterococcal bacterial protein antibodies. Antibody fragments may be produced using conventional techniques, for example, by enzymatic digestion with pepsin or papain. Where it is desired to produce recombinant antibodies according to the invention these may be produced using for example the methods described in European Patent Specification Nos 171469, 173494, 194276 and 239400.

Antibodies according to the invention may be labelled with a detectable label or may be conjugated with effector molecule for example a drug eg. an antibacterial agent or a toxin or an enzyme, using conventional procedures and the invention extends to such labelled antibodies or antibody conjugates.

The antibodies according to the invention have a diagnostic and/or preventative and/or therapeutic use. Thus for diagnostic use the antibodies may be employed to detect whether the bacterial protein is present in a host organism, to confirm whether the host has a particular Streptococcal or Enterococcal bacterial infection, and especially to test for the presence of such organisms in culture-negative endocarditis, and/or to monitor the progress of therapeutic treatment of such infections. Diagnostic methods of this type form a further aspect of the invention and may generally employ standard techniques, for example, immunological methods such as enzyme-linked immunosorbent methods, radioimmuno methods, latex agglutination methods or immunoblotting methods.

Antibodies according to the invention also have a therapeutic use in the treatment of bacterial infection due to Streptococci or Enterococci, for example, those just described and may be used alone or conjugated to an effector molecule, in the latter case to target the effector molecule, eg an antibacterial agent, to the infecting organism. For therapeutic use the antibody may be formulated in accordance with conventional procedures, for example, with a pharmaceutically acceptable carrier or excipient, eg., isotonic saline for the administration at an appropriate dosage, depending on the nature of the infection to be treated and the age of the patient.

If desired, mixtures of antibodies may be used for diagnosis and/or prevention and/or treatment, for example mixtures of two or more antibodies recognising different epitopes of a bacterial protein according to the invention, and/or mixtures of antibodies of a different class, eg., mixtures of IgG and IgM antibodies recognising the same or different epitope(s) of a bacterial protein of the invention.

The protein or any fragment, analogue, inhibitor, antibody or antigen binding fragment thereof according to the invention may be used in a method if treatment or diagnosis of the human or animal body.

The diagnostic test method may be selected from one of the group of enzyme-linked immunosorbent assay, radioimmunoassay, latex agglutination assay and immunoblot assay.

Such a protein, fragment, analogue, inhibitor, antibody or antigen binding fragment may form part of a composition for use in a method of diagnosis or treatment of the human or animal body together with a pharmaceutically acceptable carrier, diluent or excipient.

The bacterial proteins according to the invention may be prepared by a variety of processes, for example, by protein fractionation from appropriate bacterial cell extracts, using conventional separation techniques such as ion exchange and gel chromatography and electrophoresis, or by the use of recombinant DNA techniques, as more particularly described in the "Experiments" section hereinafter. The use of recombinant DNA techniques is particularly suitable for preparing substantially pure bacterial proteins according to the invention.

Thus according to a further aspect of the invention we provide a process for the production of a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of

formula (1) or a fragment or an analogue thereof, comprising the steps of (1) culturing a host organism transformed with a vector including a gene coding for a precursor of said protein and (2) recovering said protein.

Preferably the precursor cleaved in this aspect of the invention is a fusion protein comprising at least a portion of a protein produced in a transformed host organism and at least the amino acid sequence of formula (1). Such fusion proteins form a further aspect of the invention. Desirably the fusion protein includes a protein produced at a high level by a transformed host organism. Suitable such proteins include at least a portion of a chloramphenicol acetyltransferase (CAT) protein or, preferably at least a portion of the B-galactosidase protein.

According to a still further aspect of the invention we provide a DNA sequence coding for a bacterial protein or an immunogenic fragment or an analogue thereof expressed during infection due to Streptococci or Enterococci having substantially the nucleotide sequence of formula (2):

```
5'        10                  30                  50
GAATTCACCT TCTACGATGA AAATGACCAA CCAATTAATT TTGACAATGC TCTTCTTTCA
         70                  90                 110
GTAGCCTCAC TTAACCGTGA GCATAACTCT ATTGAGATGG CTAAGGATTA TAGTGGTACT
        130                 150                 170
TTTATTAAAA TCTCAGGTTC ATCCATCGGT GAAAAAAATG GCATGATTTA TGCCACAGAA
        190                 210                 230
ACCCTGAACT TTAAACAAGG ACAGGGTGGA GCTCGCTGGA CAATGTATCC AAATCGTCAG
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       250                 270                 290
CCAGGTTCAG GTTGGGATTC ATCAGATGCA CCAAACTCTT GGTACGGTGC AGGGGCCATT
       310                 330                 350
AGTATGTCCG GTCCTACGAA TCACGTTACA GTTGGTGCAA CATCTGCTAC CAATGTGATG
       370                 390                 410
TCCGTAGCAG AAATGCCTCA AGTACCTGGA AGAGACAATA CTGAAGGTAA AAGACCAAAC
       430                 450                 470
ATCTGGTACT CACTCAATGG TAAAATTCGT GCGGTTGACG TTCCGAAAAT TACAAAAGAA
       490                 510                 530
AAACCAACTC CACCGGTAGC ACCAACTGAA CCACAAGCTC CTACCTATGA AGTGGAGAAA
       550                 570                 590
CCACTGGAAC CGGCTCCAGT AGCACCAAGC TACGAAAATG AGCCAACTCC ACCAGTAAAA
       610                 630                 650
ACTCCAGATC AACCGGAGCC ATCAAAACCA GAAGAGCCAA CATATGAGAC AGAGAAACCA
       670                 690                 710
TTGGAACCAG CTCCAGTAGC ACCAAACTAC GAAAATGAGC CAACTCCACC AGTAAAAACT
       730                 750                 770
CCAGATCAAC CAGACCCATC AAAACCGGAA GAGCCAAACT ATGAGACAGA GAAACCATTG
       790                 810                 830
GAACCAGCTC CAGTAGCACC AAGCTATGAA AATGAGCCAA CTCCACCGGT AAAAACTCCA
       850                 870                 890
GATCAACCAG AGCCATCAAA ACCAGAAGAG CCAAATTATG ATCCATTGCC AACTCCGCCG
       910                 930                 950
CTAGCACCAA CTCCTAAGCA GTTGCCAACA CCACCAGCGG TGCCAACAGT TCACTTCCAT
       970                 990                1010
TACAATCGTC TATTTGCACA ACCTCAGATT AATAAAGAAA TTAAAAACGA GGATGGAGTA
      1030                1050                1070
GATATTGATC GTACTCTAGT TGCTAAGCAG TCTGTAGTGA AGTTTGAGCT GAAAACAGAA
      1090                1110                1130
GCTTTAACTG CTGGTCGTCC AAAAACAACT TCGTTTGTAT TGGTAGATCC ACTTCCAACT
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      1150                    1170                    1190
GGCTATCAGT TTGATTTGGA AGCAACCAAG GCTGCAAGCA AAGGTTTTGA AACAAGCTAT
      1210                    1230                    1250
GACAAAGCTA GTCACACTGT AACCTTTAAG GCTACTGAGG AGACCTTAGC TGCTTTCAAT
      1270                    1290                    1310
GCTGATTTGA CAAAATCCTT TGAGACTCTA TATCCAACTG TTGTTGGTCG TGTCTTGAAT
      1330                    1350                    1370
GATGGGGCGA CTTATACGAA TAACTTTACA TTGACAGTCA ACGATGCTAC TGGTGTCAAG
      1390                    1410                    1430
TCAAACATTG TTCGTGTAAC GACTCCAGGT AAACCAAATG ATCCTGACAA TCCAAATAAC
      1450                    1470                    1490
AACTACATCA AGCCTTTGAA AGTTAACAAG AACAAGCAAG GTGTGAATAT TGATGGCAAA
      1510                    1530                    1550
GAAGTTCTAG CTGGTTCAAC GAACTACTAT GAACTCACAT GGGATTTGGA TCAATACAAG
      1570                    1590                    1610
GGAGATAAAT CTTCTAAAGA AGCGATTCAA AATGGTTTCT ACTATGTGGA TGATTATCCA
      1630                    1650                    1670
GAAGAAGCTT TAACGCTTCA ACCAGAATTG GTTAAGATTC GTGATCTAGA GGGCAACCTT
      1690                    1710                    1730
GTATCAGGTA TCAGTGTTCA ACAGTTTGAT AGTTTAGAAC GTGCGCCTAA GAAGGTTCAA
      1750                    1770                    1790
GATCTGTTGA AGAAAGCAAA CATCACTGTT AAAGGTGCTT TCCAACTCTT CTCAGCTGAT
      1810
AATCCAGCTG AATTC
```

and homologues thereof.

A DNA sequence according to the invention may be further characterised in that the bacterial protein for which it encodes may be characterised by either one or

both of the following features:-

(1) It is an immunodominant conserved antigen; and

(2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection;

A DNA sequence may also be further characterised in that the protein for which it encodes is involved in binding to heart valves.

DNA with this sequence may be obtained from bacterial genomic DNA as described in the "Experiments" section hereinafter.

The DNA sequence according to this aspect of the invention may be incorporated in an expression vector using conventional techniques. Thus in a further aspect of the invention we provide an expression vector including substantially a DNA sequence of formula (2) or a homologue thereof.

The vector may be adapted for use in a given host cell by the provision of suitable selectable markers, promoters and other control regions as appropriate. Host cells transformed with such vectors form a further aspect of the invention. Suitable host organisms include bacteria (eg. E.coli), and mammalian cells in tissue culture.

The DNA sequence of formula (2) may also be used to design DNA probes for use in identifying the presence of Streptococcal and Enterococcal bacteria in the infected state and the invention extends to such DNA probes. Such probes may also be of use for detecting circulating bacterial nucleic acids, for example using a polymerase

chain reaction, as a method of diagnosing such bacterial infections. The probe may also be synthesised using conventional techniques and may be immobilised on a solid phase, or may be labelled with a detectable label.

A DNA sequence, vector, probe or inhibitor according to the invention may be used in a method of treatment or diagnosis of the human or animal body.

It is known that Streptococci and Enterococci cause endocarditis by binding to heart valves and causing damage thereto. A possible mode of action of Streptococci in binding to heart valve tissue, or prosthetic valves, has been proposed to involve pre-binding of Steptococci to extracellular matrix proteins, such as fibronectin, which leads to the adherence and colonisation of bacteria to damaged valvular surfaces (Lowrance *et al.*, J. Clin. Invest., 1990, 86: 7-13). This suggests that fibronectin acts as a tissue receptor for the bacteria. However, we show for the first time that the prior art theory on the role of fibronectin in endocarditis is incorrect. In our studies, described below, antibody against fibronectin binds to PAc and is neutralised by prior crossabsorption with fibronectin indicating that PAc acts like fibronectin binding to damaged heart valves directly, and not via fibronectin. This molecular mimicry is the probable mode of action of PAc.

Thus the invention also provides fibronectin or an immunogenic fragment thereof or an analogue thereof or an antibody thereto or an antigen binding fragment thereof for use in a method of treatment or diagnosis of the human or animal body for infection due to Streptococci or Enterococci.

Additionally, as described below in the "Experiments" section, we have shown for the first time that serum from patients with endorcarditis due to S.oralis, S.gordonii and S.sanguis infection have antigen of approximately 85 kDa which reacts with a mouse monoclonal antibody specific to heat shock 90 molecules. We have also shown that in a mouse *S. oralis* infection model with death as an end point, antibody specific to HSP90 shows a statistically significant increase in survival. This shows that this antigen is in the HSP 90 group. Thus antibodies against HSP 90 may be used for the diagnosis and treatment of infections caused by S.oralis, S.gordonii and S.sanguis.

Thus the present invention provides antibodies specific to HSP 90 or immunogenic fragments or analogues thereof for use in a method of diagnosis or treatment of the human or animal body of infection due to streptococci or enterococci due to any one of the group of S.oralis, S.gordonii and S.sanguis.

The invention will be further apparant from the following "Experiments" section which exemplifies the invention.

Experiments

1. Characterisation of the Antibody Response

Sera was available from the following cases:

12 cases of septicaemia due to Streptococcus oralis,

14 cases of endocarditis due to Streptococcus gordonii,

2 cases of endocarditis due to Streptococcus oralis,

2 cases of endocarditis due to Streptococcus sanguis,

20 control sera from patients having no clinical evidence of endocarditis who were non-neutropenic, and

20 control sera from patients having no clinical evidence of endocarditis who were neutropenic.

2. Species Identification

Using the above sera, the causative organisms were identified, when available, according to the scheme of Beighton *et al.*, J. Med. Microbiol., 1991, 35: 367-372. Specifically, isolates from 6 of the cases of S.oralis were available and identified as S.oralis by JM Hardie (personal communication). The others were aesculin-negative and raffinose-negative -viridans streptococci eliminating S.gordinii (100% aesculin positive), S.mitis (100% raffinose positive) and making it unlikely to be S.sanguis (75% aesculin positive and 75% raffinose positive). In the 14 cases of S.gordonii and 2 cases of S.sanguis endocarditis, isolates from 3 were positively identified as S.gordonii. All the other isolates were unobtainable but the original API

Streptococcal profile showed they were aesculin positive. This would eliminate S.mitis and make it unlikely that they were S.oralis (18% aesculin positive). They were then subdivided into either S.gordonii or S.sanguis according to whether the more dominant IgM response on immunoblot was against S.gordonii or S.sanguis. The cases of S.oralis endocarditis were identified according to Beighton *et al.* 1991.

3. Source of Strains for Immunoblotting

Using the protocol outlined by Burnie *et al.*, J. Clin. Pathol., 1987, 40: 1149-1158, antigenic extracts were made from S.sanguis NCTC 7863, S.oralis NCTC 7864 and S.gordinii NCTC 7868.

4. Immunolblotting

Immunoblotting using the above strains was then carried out as described in Burnie *et al.*, 1987.

The results of immumoblots of the patients sera described above is summarised in Table 1 (S.oralis), Table 2 (S.gordonii) and Table 3 (S.sanguis) and in Figures 1 to 5.

Figure 1 shows the immunoblot for S.oralis septicaemias. Paired IgMs and paired IgGs covering pre and post sera from 3 of the cases showing the antibody changes.

Figure 2 shows the immunoblot of S.gordonii endocarditis. Anti-fibronectin antibody

against S.gordonii NCTC 7868 in Track 1, crossabsorbed against fibronectin in Track 2. Tracks 3-8 show IgM and IgG respectively in 3 cases of S.gordonii endocarditis.

Figure 3 shows 5 further case of S.gordonii endocarditis versus S.gordonii antigen Tracks 1/2, 3/4, 5/6, 9/10 and 11/12. Tracks 7/8 show pre-treatment of antibody levels of the same case as tracks 9/10 showing increased IgM to bands at approximately 85 KDa and IgG to bands at approximately 180, 58, 56 and 52 Kda.

Figure 4 shows the immunoblot of S.oralis endocarditis, Tracks 3/4 IgM and IgG, antifibronectin antibody crossreacting with 180 KDa band of S.oralis, Track 1 and effect of crossabsorption with fibronectin, Track 2.

Figure 5 shows the immunoblot of S.sanguis endocarditis, showing both IgM (track 3 and 5) and IgG (tracks 4 and 6) respectively of both cases, antifibronectin antibody crossreacting with the 120 KDa band of S.sanguis, Track 1 and the effect of crossabsorption with fibronectin, Track 2.

Comments

(a) S.oralis septaceamia

IgM and/or IgG against band at approximately 180 KDa when recovering from infection.

(b) S.gordonii endocarditis

Additional IgG was detected against bands at approximately 185 (1 case), 165 (2 cases), 155 (1 case), 140 (1 case), 132 (1 case), 110 (1 case), 94 (1 case), 61 (1

case), 50 (1 case) and 45 KDa (1 case). Additional IgM was detected against the 45 KDa band (1 case). The majority of cases has IgM and all IgG against the bands at 85 and 180 KDa from S.gordonii NCTC 7868. Other immunodominant bands included those at approximately 65 and 47 KDa.

(c) S.oralis endocarditis

case 1: Had additional antibody as follows. IgM against bands at approximately 180, 140 and 65 KDa and IgG agaist bands at approximately 180, 140, 120, 58, 51, 46 and 35 KDa. Both cases had IgM and IgG against the bands at 85 and 180 KDa from S.oralis NCTC 7864.

(d) S.sanguis endocarditis

Both patients produced antibody (IgM and IgG) against bands at approximately 120 and 85 KDa from S.sanguis NCTC 7863.

(e) S.sobrinus/S.mutans endocarditis

Immunoblotting demonstrated that patients with endocarditis had antibody (IgM and IgG) against three bands at approximately 185, 200 and 220 KDa.

Additionally the bands at 85 KDa (S.oralis NCTC 7864, S.gordonii NCTC 7868 and S.sanguis NCTC 7863) all react with a mouse monoclonal specific to heat shock 90 molecules (versus LKVIRK, see our previous patent application Nos WO 92/01717, WO 91/00351 and GB 2270076) showing that this molecule is in the HSP 90 group (Figure 6), Track 1 S.oralis NCTC 7864, Track 2 S.gordonii NCTC 7868, Track 3 S.sanguis NCTC 7863, Track 4 clinical isolate of S.oralis.

5. Cloning of the immunodominant antigen of S.sobrinus and S.oralis

5.1 S.sobrinus cloning

(i) DNA isolation and lambda ZAPII library preparation.

Strain S.sobrinus Manchester University Collection of Bacteria No.263 (MUCOB 263), was used as the source of S.sobrinus. Initially, the MUCOB 263 organism was identified as an S.mutans, a fact reflected in the aboved mentioned publication. However, a more detailed testing of theis strain revealed that MUCOB 263 is biochemically more similar to S.sobrinus (Professor D. Bratthall, personal communication). To avoid future confusion, the organism has now been renamed S.sobrinus MUCOB 263. Bacterial cells were grown overnight at 37°C, with shaking, in brain-heart infusion broth containing 0.2% glucose and 40mM D,L-threonine (Sigma). The bacteria were harvested by spinning at 5,000 rpm for 10 mins before being washed, and resuspended in 12.5ml of 0.02 M Tris pH 8.2. Then 25 ml of 20 M polyethylene glycol (PEG) 24% in distilled water was added and mixed. Lysozyme was added at 34.6 mg/ml equivalent to 100µg/unit obtained by measuring the OD600 of a tenfold dilution of bacterial cultures. After 1 hour at 37°C, the spheroblasts formed were spun down at 5,000rpm for 10 mins and thoroughly resuspended in 50 ml of 10 mM Tris-Cl pH 8.0, 1 mM EDTA (TE). The spheroblasts were lysed with 5.5 ml of 10% sodium dodecyl sulphate (SDS) at 60°C for 15 mins. The DNA was purified by standard procedures (Maniatis *et al.*, Molecular Cloning - A laboratory manual, 2nd edn. Cold Spring Harbour, New York, 1989), RNAase A (sigma) treated (after preboiling the RNAase at 100°C for 15 mins), proteinase K treated, phenol:chloroform:isoamylalcohol (25:24:1) extracted, and ethanol precipitated. Plasmid DNA was removed by ethanol precipitation in the presence of 0.3 M ammonium acetate pH 5.2. Further purification was obtained

by dialysis against TE over 36 hrs at 4°C.

The DNA was mechanically sheared, EcoRI linkers added, fractionated and inserts ligated into lambda ZAPII vector arms. The library had an insert size range of 2kb-7kb.

(ii) Antibody Screening

Serum was taken from a patient with endocarditis and used for antibody screening. Escherichia coli XL1 - Blue cells were infected at approximately 3,000 pfu/85mm on L broth agar (Bacto-tryptone 10 g/l, yeast extract 5 g/l, sodium chloride 10 g/l, maltose 2 g/l, bacto-agar 15 g/l). Plaques were transferred to nitrocellulose filters (0.45 µm pore size, Sartorius AG, Gottingen, Germany), impregnated with 10 mM isopropyl B-D-thiogalactopyranoside (IPTG), at 37°C for 2 hrs, after a 42°C incubation for 3 hrs. These filters were blocked overnight at 4°C with 3% bovine serum albumin (BSA - Sigma) in buffered saline (150 mM NaCl, 10 mM Tris). Patient serum, diluted one hundredfold in 3% BSA, was added to the filters and incubated at room temperature for 2 hrs, the filters then being washed for 30 mins in washing solution (150 mM NaCl, Tween 20, 0.05%), before the second antibody, a thousandfold dilution of anti-human IgG alkaline phosphatase conjugate (Sigma) in BSA 3% was added. After 1 hr at room temperature, the filters were again washed and stained with equal volumes of naphthol ASMX phosphate (Sigma, 0.4 mg/ml in distilled water) and Fast Red TR salt (Sigma, 6 mg/ml in 0.2 M Tris pH 8.2) (the Fast Red stain). Positive plaques were transformed to 1.5 ml tubes containing 200 µl of SM (100 mM sodium chloride, 50 mM Tris-Cl pH 7.5, 10 mM magnesium sulphate, gelatine, 0.0001%), and 2 to 3 drops of chloroform. Plaque

purification was performed by the above method.

(iii) Antigen-directed antibody selection

Two 100 µl aliquots of resuspended E.coli XL1-Blue cells were infected with a dilution of a high titre stock of purified positive phage, to give 5,000 pfu / 85 mm L broth agar plate. Phage adsorption occured at 37°C for 30 mins. The adsorption mixes were added to 2.5 ml aliquots of L broth agarose 0.8%, mixed, and poured onto L broth agar plates. These plates were incubated at 42°C for 2.5 hrs, before nitrocellulose filters (0.45 µm pore size, Sartorius), presoaked in 10 mM IPTG and dried at room temperature for at least 1 hr, were added and incubated at 37°C overnight. Each filter was then washed 3 times over 30 mins in Tris-buffered saline (150 mM sodium chloride, 10mM Tris-Cl pH 7.2), and blocked overnight at 4°C in 3% BSA. A tenfold dilution of patient serum in BSA 3% was added to the filters and incubated, with shaking, at room temperature for 3 hrs. This serum was removed, called the depleted serum and stored at 4°C. The filters were washed 5 times over 100 mins with Tween 20-Tris-buffered saline (150 mM sodium chloride, 10 mM Tris-Cl pH 7.2, Tween 20, 0.05%), and once with salt - Tween 20 solution (150 mM sodium chloride, Tween 20, 0.05%). The bound antibody was eluted by adding 5 ml of glycine saline buffer (150 mM sodium chloride, 200 mM glycine-Cl pH 2.8), and shaking at room temperature for 30 mins. The buffer was aspirated into a bijoux, containing Tris 0.04g per 100 µl, mixed, termed the eluted antibody and stored at 4°C. A Western blot of S.sobrinus MUCOB 263 was performed essentially by the immunoblot method above with the original serum, depleted serum and eluted antibody being used as the primary antibodies. The antigen was the supernatant of the strain following crushing in an X-press. The secondary antibody was a

thousandfold dilution of anti-human IgM and IgG alkaline phosphatase conjugates in 3% BSA, and the stain was the alkaline phosphatase stain.

Results

Six antibody positive clones were isolated and purified from the 60,000 pfu initially screened with culture-positive endocarditis patient serum. Antigen-directed antibody selection showed that the cloned, expressed sequence contained epitopes shared by S.sobrinus as the eluted antibody bound to the approximately 185 KDa antigen.

(iv) In vivo excision of DNA insert

A 10 ml overnight culture of E.coli XL1-Blue cells grown at 37°C in L broth, containing tetracycline 125 µg/µl, was spun down at 2,000 rpm for 10 mins, resuspended in 4 ml of 10 mM magnesium sulphate, and stored at 4°C. In a 50 ml plastic conical tube, 200µl of the resuspended E.coli XL1-Blue cells, 180µl of high titre antibody-positive phage (1.78 x 108/85 mm plate approx) and 1 µl of R408 helper phage were combined and incubated at 37°C for 15 mins. (Helper phage and E.coli XL1-Blue cells from CLONTECH). Then, 5 ml of 2 x YT media (sodium chloride 5 g/l, yeast extract 10 g/l, bacto-tryptone 16 g/l pH 7.0) was added to the dual-infected bacteria and incubated, with shaking, at 37°C for 3 hrs. The cells were killed by heating at 70°C for 20 mins and ruptured by vortexing for 5 mins. The cells were spun at 4,000 rpm for 5 mins, and the supernatant containing pBluescript SK(-) phagemid decanted and stored at 4°C. The phagemid was propagated by adding 10 µl of the phagemid solution to 200 µl of resuspended E.coli XL1-Blue cells, incubating the culture at 37°C for 15 mins, and

plating 1 µl, 25 µl, 75 µl and 100 µl aliquots onto L broth agar plates, containing ampicillin 50 µg/ml.

(v) Plasmid DNA preparation and denaturation

The pBluescript SK(-) plasmid DNA was purified from the bacterial colonies with the Magic (RTM) Minipreps DNA purification System (Promega) and alkaline denatured by the method of Maniatis *et al.*, 1989. Hence, 8 µl of solution, containing DNA 1.5 - 200 µg was added to 2 µl of 2 M sodium hydroxide, briefly vortexed and spun, and left at room temperature for 10 mins. Then 3 µl of 3 M sodium acetate (pH 4.8), 7 µl of distilled water, and 60 µl of -20°C absolute ethanol were added and the DNA precipitated at - 70°C for 30 mins. The precipitate was pelleted at 13,000 rpm for 1 min, washed with -20°C ethanol 70%, and vacuum-dried. The dried pellets were stored at -20°C.

(vi) DNA sequencing

DNA sequencing was performed by the two-step chain-termination method, with Sequenase (R) Version 2.0 (Cambridge Bioscience). The annealing step was carried out at 66°C for 2 mins, the labelling at 22°C for 4 mins, and the termination at 38°C for 5 mins. Areas containing secondary structure were resolved by the substitution of dITP for dGTG. Sequencing reactions were run on acrylamide gels 6% for 11, 8, 5 and 2 hrs at 46-51°C.

(vii) Subcloning with the TA cloning (RTM) system

The phagemid clone did not carry the 5' end of the cloned gene, therefore it was decided to subclone the 5' end by Polymerase Chain Reaction (PCR), followed by TA cloning (RTM). The following PCR reagents were added to each of the 2 tubes containing 0.0 µg, 0.7 µg of S.sobrinus MUCOB 263 genomic DNA, with distilled water to give 64.3 µl total volume; 5.3 µl or 0.1 µg/µl Primer 1 (CAGTCTCCGTCCCAACGACTGCG), 4.4 µl of 0.1 µg/µl Primer 2 (GCTCCTCTTGTGACATGGTC), 10 µl of 10 x Taq buffer (Northumbria Biochemicals Ltd., Northumberland (nbl), and 16 µl of dNTP's (12.5 µl of each of dATP, dGTP, dCTP, dTTP (Promega), added to 950 µl of deionized water). Then, 60 µl of mineral oil was carefully layered on top of the PCR reactions, the DNA denatured at 95°C for 10 mins, and 2.5 Weiss Units of Taq (nbl) added. The PCR conditions were 94°C for 1.5 mins, 50°C for 1.5 mins, and 72°C for 3 mins for 30 cycles, before a final long extension of 72°C for 10 mins. The PCR products (30 µl) were run on a TBE gel 0.8%, at 50 volts for 2 hours, alongisde 4 µl of EcoR1 - Hind III cut lambda DNA markers (nbl).

Insert DNA was subcloned with the TA Cloning (RTM) System Version 1.3 (InVitrogen Corporation, British Biotechnologies Ltd, Oxon) as specified in the manufacturers protocol. Overnight, 12°C, ligation reactions containing 3.0 µl of a fourfold dilution, in distilled water, of the 0.7 µg PCR reaction, 4.7 µl of doubling dilution of the 0.7 µg PCR reaction, and a negative control were performed. In the transformation, white recombinant colonies were picked after overnight 37°C incubation, and twice replated on kanamycin (50µg/ml)/ 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside (X-Gal-25 µl of 40 mg/ml solution/plate) to achieve pure and stable subclones. Plasmid DNA was obtained, purified, denatured and sequenced. The origin of the cloned DNA from S.sobrinus was confirmed by Southern Blotting. The full

sequence obtained in shown in Table 4.

5.2 S.oralis cloning

This was done essentially by the methods described above. The differences were that the vector was lambda gt11 and the DNA source was a clinical isolate of Streptococcus oralis. The library was a genomic DNA library partially digested with EcoR1. Ten positive clones were identified and one of these subcloned by the TA Cloning (RTM) System. The origin of the clone from S.oralis was confirmed by Southern Blotting. Antigen directed antibody selection showed that it had epitopes which cross-reacted with the 180 KDa antigen of S.oralis (Figure 7). Tracks 1 and 3 show the original serum from two patients with antibody against S.oralis, and tracks 2 and 4 show the eluted subcomponent binding to the band at 180kDa. The sequence of the clone is given in Table 5.

Comparison of the Sequences

The sequence obtained from S.sobrinus had a 99.2% homology to the SpaA antigen of S.sobrinus and a 68.4% homology to the PAc antigen of S.mutans. The S.oralis peptide carried a 76.2% homology over 605 amino acids with the S.mutans PAc protein precursor and 73.8% homology over 606 amino acids with both the S.sobrinus SpaA protein precursor and the S.mutans surface antigen I/II precursor. Both sequences contain a three tandem repeat motif of 39 amino acids. Six peptides were derived from these and used to epitope map this communal sera.

Epitope Mapping

Epitope mapping of the antigen cloned from S. oralis was carried out according to the protocol outlined by Geysen *et al.*, J. Immunol. Methods, 102: 259-274 (1987) and references therein. In this epitope mapping, a complete set of overlapping nonapeptides was synthesised for the cloned S. oralis antigen. Peptide 1 covered residues 1-9, peptide 2 covered residues 2-10 and peptide 3 covered residues 3-11 etc. The epitopes were tested against various patient sera. All of the sera were examined at a 1 in 200 dilution for IgG and recording was stopped after 30 minutes. The sera examined were:

(1) Viridans endocarditis (n=8) (*S. sanguis* n=2, *S. oralis* n=2, *S. gordonii* n=4)

(2) *S. oralis* septicaemia n=5

(3) *S. mutans* endocarditis (n=2)

(4) *E. faecalis* endocarditis (n=2)

(5) Negative control (n=5)

Epitopes were defined as those peptides which:

(1) were positive in at least 3 wells

(2) had an optical density of each well which was at least double the negative control value and in the majority of wells greater than 0.8

This epitope mapping identified a total of 9 epitopes (see table 10) - NFKQGQG, RQPG, SWYGAG, GKIRAV, RLFAQPQ, AGRPK, PTGYQFD, YPTVV and LLKKA.

After the initial epitope mapping, each individual serum was then reexamined for positivity with each well (see table 11).

6. Preparation of Immunodominant Epitopes from Cloned S.oralis

Of the synthetic peptides (see 5.2; "Comparison of the Sequences"), peptides 1-4 cover the sequence of S.oralis as short peptides of 15 amino acid length. Peptides 5 and 6 covered the S.sobrinus area which differed. These sequences are listed (Table 6) and illustrated (Figure 8).

The six synthetic peptides were produced by Cambridge Biochemicals Ltd., Nantwich and dissolved in a double-distilled water to give a final concentration of 2 mg/ml. Aliquots, 0.5 ml, were stored at -70°C and after one freeze-thaw cycle, stored at -20°C.

The wells of six plastic microtitre plates (Falcon (R) 3912, Microtest (RTM) Flexible Assay Plate, 96 flat-bottom wells - Becton Dickinson & Co, Fred Baker, Liverpool), one for each peptide, were coated with 200 µl of the appropriate 10 µg/ml synthetic peptide solution diluted in phosphate buffered saline (one tablet dissolved in 100 ml distilled water, Oxoid, Unipath Ltd, Basingstoke; PBS). The peptides were added and left at 4°C overnight to coat the wells. Excess peptide was then removed by washing the microtitre plates five times in PBS with a Nunc-Immuno Wash (RTM) (InterMed, Denmark). The primary antibody, 200 µl of a 1/10 dilution in 3% BSA of sera was added and left at room temperature for 1 hour to allow antigen-antibody binding. After washing five times in PBS, the wells were filled with 200 µl of one of

i) a 1/1000 dilution of goat anti-human IgM peroxidase conjugate in 3% BSA (Sigma),

ii) a 1/1000 dilution of goat anti-human IgG peroxidase conjugate in 3% BSA (Sigma), or

iii) with PBS.

The conjugates were left to react at room temperature for 1 hour. The plates were then washed as before and 200 µl of ABTS stain was added to each well (three tablets of 2.2' amino-bis(3-ethylbenzthiazoline-6- sulfonic acid); diammonium salt; Sigma) and 160 µl of 30% w/w hydrogen peroxide solution; Sigma, in 60 ml of 125 mM disodium hydrogen orthophosphate buffer brought to pH 4.0 with 1M citric acid). This ABTS stain was prepared just prior to use. The signal strength for each well was then read at 405 nm at 5, 10 and 15 mins after the start of the reaction with a microtitre plate reader (Titertek Muliskan (10) PLUS MKH, Labsystems, Finland) attached to an impact dot matrix printer (Panasonic KX-P1081, Panasonic Matsushita Electric Industrial Co Ltd, Osaka, Japan).

To determine which of the six peptides representing the S.sobrinus/S.oralis antigenic region which produced the most specific and strongest positive signal, each of the peptides was used in an indirect ELISA.

All peptides were screened against a panel of 5 sera (single sera from cases of S.mutans endocarditis, S.oralis endocarditis, S.oralis septicaemia, S.lactis endocarditis and S.aureus endorcarditis).

All 6 peptides were recognised by the sera from the cases of endocarditis due to S.mutans and S.oralis. They had much lower Optical Densities with the sera from the septicaemia due to S.oralis and the control sera from cases of endocarditis due to S.lactis and S.aureus. Peptide 1 showed the highest OD (IgG) in S.mutans endocarditis and was selected for further studies (Table 7).

Indirect ELISA

As a result of the epitope mapping and of the preparation of immunodominant epitopes from clones S. oralis (see above), three peptides were investigated further in indirect ELISA tests. These peptides were YEVEKPLEPAPVAP (Peptide 1), TMYPNRQPGSGWDSS (contains the epitope RQPG - epitope numbers 74-79; Peptide 7) and WYSLNGKIRAVDVPK (contains the epitope GKIRAV - epitope numbers 144-147; Peptide 8).

Sera Tested

Sera from cases of endocarditis due to S.mutans (2), S.oralis (3), S.gordinii (10), S.sanguis (2), E.faecalis (11), S.bovis (8), S.agalactiae (1), S.lactis (1), S.pneunoniae (4), Group G Streptococcus (2), S.aureus (2), Coagulase Negative Staphylococci (6), Candida albicans (1), Candida parapsilosis (2) and E.coli (1), septicaemias due to S.oralis (8), E.faecalis (7), E.faecium (2) and a brain abscess due to S.milleri. Further controls were sera from patients with SLE (3) and neutropenic leukaemic patients with no evidence of streptococcal infection (20).

The optical densities are given in Table 8 for Peptide 1, and in Table 8a for Peptides 1, 2 and 3.

Results

In summary, a raised IgG was specific to patients with Steptococcal endocarditis and a raised IgM specific to endocarditis due to Streptococcus oralis/gordonii/ sanguis/mitis.

If a cut off point of 0.6 is taken for the Optical Densitivity for IgG and 0.4 for IgM then all cases of S.mutans (patient 1), S.oralis (patient 4), S.gordonii (patients 6-14) and S.sanguis (patients 16 and 17) endocarditis fulfilled one or other of these criteria. All other sera were classified as negative. All controls were negative except for a raised IgG in cases of endocarditis due to E.faecalis, S.bovis and Group G streptococci (Table 9).

This data proves the value of the test in culture positive endocarditis. It could also be extended to cover culture negative cases.

One treatment of IgM fell in cases 6, 13 and 15 showing that a falling IgM was a marker of successful therapy.

An analysis of the overall results (see tables 12 and 13 below) showed that the tests performed using peptide 1 gave the most accurate and specific results (100% specificity), but were the least sensitive (50% sensitivity). Tests wth the other two

peptides were more sensitive, but less specific and gave more false positives. This suggests that although each of the peptides could be usefully used individually, a combination of tests using more than one peptide would allow for an overall test which was both highly sensitive and highly specific.

Table 12

	Peptide 1		Peptide 7		Peptide 8	
	IgM	IgG	IgM	IgG	IgM	IgG
True Positives	6	5	12	5	12	10
False Positives	0	1	28	15	25	21
True Negatives	42	41	14	27	17	21
False Negatives	6	7	0	7	0	2

(For raw data see table 8a)

Table 13

	IgM Peptides			IgG Peptides		
	1	7	8	1	7	8
Sensitivity	50%	100%	100%	41.5%	41.5%	83%
Specificity	100%	33%	40%	98%	64%	50%
Efficiency	89%	48%	53%	85%	59%	57%

7. Fibronectin Binding Studies

In order to test for fibronectin binding activity in the cloned proteins, each

of the peptides 1-6 was reacted in an indirect ELISA according to the previous protocol.

The indirect ELISA produced OD values greater than 2 for all six peptides regardless of whether fibronectin was added to the sandwich. This implied that the polyclonal serum (anti-fibronectin 0.5 mg/ml Sigma [F1509]) reacted with the peptides directly. This serum was then immunoblotted at a dilution of 1 in 40 and again after crossabsorbing, 100 µl of 1 mg/ml antibody, with 100 µl of 1 mg/ml of fibronectin (F2006 Sigma) at 37°C for 30 mins. This showed that the antiserum reacted specifically with the 180 kDa antigen of S.oralis, 180 kDa antigen of S.gordonii and 120 kDa antigen of S.sanguis. This is illustrated by Figure 2 tracks 1 and 2 (S.gordonii), Figure 4 tracks 1 and 2 (S.oralis) and Figure 5 tracks 1 and 2 (S.sanguis). Figure 9 shows the antibody crossreacting with the 185 kDa antigen of S.sobrinus (Track 1) and the 180 KDa antigen of S.oralis (clinical isolate) (Track 3) and the elimination of the crossreactivity by prior absoroption with fibronectin (Tracks 2 and 4).

This is with the previously identified immunodominant antigens of these microorganisms and implies that they act as a mimic for fibronectin. This appears to be the mechanism by which these streptococci bind to heart valves. A comparison of the sequences of the peptides with human fibronectin showed subtantial homology (GCG pogramme Gap; Sequence of fibronectin by Kornblitt *et al.* EMBO J. 4: 1755-1759, 1985). The most homologous region of the fibronectin molecule was a 69 residue sequence beginning at residue 750 of the fibronectin protein. The percentage similarity and identity values were 48.7% and 35.9% respectively (Figure 10).

8. Human Recombinant Antibodies

A library of immunoglobulin heavy and light chain variable (V) genes was prepared from the peripheral blood lymphocytes of a patient with infection due to a viridans streptococcus (blood culture positive) septicaemia following an oesophagectomy who recovered on amoxicillin and gentamicin. Screening of this library by immunoblot against S.oralis NCTC 7864 showed recombinant antibody against the band at 180 KDa (Figure 11, tracks 5 and 6). The pre and post IgM (Tracks 1 and 2) and IgG (Tracks 3 and 4) from a patient recovering from septicaemia due to S.oralis is shown for comparison.

The library was produced essentially as described by Marks *et al* (J. Mol. Biol., 1991, 222: 581-597) using the pCANTAB 5 vector, which is now commercially available as part of a kit from Pharmacia (Milton Keynes, UK). The heavy and light chain V genes, obtained from cDNA prepared from the mRNA of peripheral blood lymphocytes of a patient recovering from a viridans streptococcus (blood culture positive) septicaemia, were randomly combined and subcloned into Not I/Sfi I digested pCANTAB 5. The resulting single chain Fv fragments (ScFv), expressed on the surface of phage, were enriched by panning four times against the specific synthetic peptide epitopes YEVEKPLEPAPVAPS, TMYPNRQPGSGWDSS and WYSLNGKIRAVDVPK (Peptides 1, 7 and 8). Following the fourth panning, twenty clones (from each of the last pannings) were:

1. Bst1 fingerprinted to establish the degree of focussing of the panning procedure; and
2. Examined in an indirect ELISA against the original peptide (as described

in detail previously). Conditions were:-

Recombinant antibody: neat

Phage specific monoclonal : 1 in 2,000

Antimouse Horse Radish Peroxidase 1 in 1,000

100ml of Peptide applied to well in PBS at 10ug/ml and incubated overnight at 4^0C.

Results

Peptide 1

20 clones selected were identical on Bst1 fingerprinting. Indirect ELISA varied from 0.25-0.30 (control 0.17). Two clones (PAC 1 and PAC 2) selected for animal work.

Peptide 7

16 clones produced 6 types. One type (Type A) was present in 7 out of the 16 clones and was the only type to produce a positive reading in the indirect ELISA (0.276-0.318) (control 0.17). One clone (Clone 3) selected for animal work (PAC 3).

Peptide 8

16 clones produced 10 types. Two of these, A and B were represented by 3 and 5 clones respectively. None of these clones produced a positive ELISA result. Five of the 8 clones produced a positive ELISA result (range 0.235-0.304 control 0.138) and a unique Bst1 fingerprint. One of these (Clone 7) was selected for animal work (PAC 4).

In order to test the efficacy of the selected clones specific to Peptides 1-3 in treating *S. oralis* and vancomycin-resistant *E. faecium* infections, a set of experiments with PAC 1-4 and other controls was performed.

Experiment 1

This experiment was an acute streptococcal infection model using Balb/c mice with an end point of death. Conditions were:

S.oralis dose: 5.7×10^9

Phage dose: 5×10^8 pfu/ml

Antibody given first, followed by *S.oralis* 2 hours later.

Table 14 (results of Experiment 1)

SURVIVORS				
Antibody	No. of mice	4 hours	24 hours	48 hours
Control: no antibody M13K07 phage	15	7	3	1
DEPAGE	13	3	3	2
B3.7	13	6	6	6
B3.14	8	1	1	1
PAC 1	9	9	2	2
PAC 2	17	8	8	8
PAC 3	15	6	6	6
PAC 4	15	2	2	2

DEPAGE - antibody against a Candidal specific carboxy-end HSP90 antigen (sequence DEPAGE) which acts as an irrelevant phage and therefore a control.

Fisher exact 2 tailed P value showed statistical significance for B3.7 at 48 hours (P 0.03), PAC 1 at 2 hours (P 0.0095) and PAC 2 at 48 hours (P 0.02). B3.7 is a recombinant

antibody specific to the HSP90 stress protein, suggesting that antibodies specific to the HSP90 protein may be used to diagnose and treat *S. oralis* and possibly other streptococcal and enterococcal infections.

Experiment 2

This was a chronic streptococcal infection model using CD1 mice and a colony count of spleen and kidney was performed at the various end-points. Conditions were:

S.oralis dose 2.5×10^9

Phage dose 5×10^{10} pfu/ml

S.oralis given first, antibody given 24 hours later.

Positive organ count > 10^4/g/ml

S = Spleen, K = Kidney

S + K = combined spleen and kidney results

5 mice sacrificed on days 4, 7 and 12

Repeat antibody injection at day 10.

Table 15 (results of Experiment 2)

	Day 4			Day 7			Day 12		
	S	K	S+K	S	K	S+K	S	K	S+K
Control:no antibody M13K07 phage	4	4	8	0	0	0	1	0	1
B3.14	1	2	3	0	0	0	0	0	0
PAC 2	0	1	1	1	1	2	1	0	1
PAC 3	1	2	3	0	0	0	0	0	0

No phage recovered after 24 hours post injection, all mice blood cultures negative.

Fisher exact 2 tailed P value showed statistical significance for PAC 2 ($P = 0.005$) when spleen and kidney results were combined from day 4.

Experiment 3

This was an infection model with CD1 mice using high level vancomycin-resistant *E. faecium*. Conditions were:

E. faecium dose: $3 \times 10^{10/}$ ml

Phage dose: 5×10^{10} pfu/ml

E. faecium given first, antibody given 24 hours later.

Positive > 10^4 g/ml. S = spleen, K = Kidney

S + K = Spleen + Kidney.

Spontaneous deaths cultured at day 2

5 mice sacrificed on day 4

Remaining mice 3 for the M13K07 control and 5 for the PAC 2 antibody, sacrificed at day 7.

Table 16 (results of Experiment 3)

	Day 2 (spontaneous deaths)	Day 4 (n=5)			Day 7 (n=3 control) (n=5 PAC 2)		
		S	K	S+K	S	K	S+K
Control no antibody: M13K07 phage	6[a]	2	5	7	0	0	0
PAC 2	4[a]	2	3	5	0	0	0

[a] all spleens and kidneys cultured and positive > 10^4 /gl/ml

Total positive Control : 19/22

PAC : 13/18

This produced by combing the spleen and kidney results obtained from the spontaneous deaths (day 2) with 5 mice (day 4) sacrificed assuming > 10^4 organisms /g/ml is positive. This suggests PAC2 may have some activity agianst vancomycin-resisitant *E.faecium.*

Experiment 4

This was an acute streptococcal infection model using Balb/c mice infected with vancomycin-resistant *E. faecium*. The end-point was death. Coonditions were:

Organism: high level (>256 mg/l) vancomycin-resistant *E. faecium*

E. faecium dose: 5 x 10^{10} /ml

Phage dose: 5 x 10^{10} pfu /ml

Antibody given first, followed by *E.faecium* 2 hours later.

Table 17 (results of experiment 4)

Antibody	No of mice	Survivors 4hours	24hours	48hours
Control no antibody M13K07 phage	15	10	5	1
PAC2	15	12	8	6

Fisher exact 2 tailed P value showed statistical significance for Pac 2 at 48 hours (p 0.02)

Table 1 Immunoblot testing of the *S.oralis* NCTC 7864 antigen against patient sera.

M.Wt. of *S.oralis* antigen band	Patient Sera: Endocarditis	Sequential sera S.oralis. septicaemia	Controls

n = 12

	S.oralis Endo-carditis	*S.gordonii* Endo-carditis	*S.sanguis* Endo-carditis	Constant Septicaemia	Inc. x 2 or App.	Controls Neutropenic	Controls Non-Neutropenic
	n = 2	n = 4	n = 2			n = 20	n = 20
	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG
192	1 1	2 10	1 2	1 3	1 5	4 6	2 1
185	1	4	1 1	1	1 3	2 3	1 2
180	2 2	4 13	1 2	2	9 10	1 3	4
155	1	9	1	4		2	
140	1 2	2 6		3 8	1 2	2 8	1 2
105	1	1 5	1	1		2 2	1 1
85	2 2	4 11	1 2	8	1 1	3 3	1 1
76	1	1 3	1	1 5	2 1	2 2	
65	1	3			1		
51	1	6 3	1	11	2	1 11	6
46	2	3		1	1	1	
35	1	1	1				

Inc = Increased x 2 App = Appeared

Table 2 Immunoblot testing of the *S.gordonii* NCTC 7868 antigen against patient sera.

M.Wt. of *S.oralis* antigen band	Patient Sera: Endocarditis				Sequential sera S.oralis. septicaemia n = 12		Controls					
	S.oralis Endo-carditis		*S.gordonii* Endo-carditis		*S.sangius* Endo-carditis		Constant Septicaemia		Inc. x 2 or App.		Controls Neutropenic		Controls Non-Neutropenic	
	n = 2		n = 4		n = 2						n = 20		n = 20	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
185		2	2	5			1	7						
180		2	12	14	2	2		6				5	1	2
105		1		1			2	4			1	3		1
85	2	1	12	14	1	1	1	10					1	1
80			5	12	1	1	1	9			1	11		4
76			5	7	1	1					1	10	1	3
72	1	1	5	7		1				1	1	4	1	1
70		1	4	5	1	2		7						
65			9	13	1	2	1	4				1		1
58			1	9										
56			2	9		1								
52			1	8		2								
47		2	12	14	1	1		10		2	1	15	2	2
40			5	8	2	1		8		1		1		
37			8	8	2	2								
35			3	10	1	1		2		1				
33			2	9										
30			1	11	2	2								
28			2	7		1								

Inc = Increased App = Appeared

Table 3 Immunoblot testing of the *S.sanguis* NCTC 7863 antigen against patient sera.

M.Wt. of *S.sanguis* antigen band	Patient Sera											
	Endocarditis						Septicaemia					
	S.oralis Endo-carditis		*S.gordonii* Endo-carditis		*S.sanguis* Endo-carditis		*S. oralis* Septicaemia		Controls Neutropenic		Controls Non-Neutropenic	
	n = 2		n = 14		n = 2		n =9		n =20		n = 20	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
120		2	4	14	2	2		1	3	3		4
85		1		2	2	2		8	3	3	2	4
83					2	2						
81			2	8	2	2						
75	1	1	4	2		2	2	5	3	15		8
59	1	1		2	1						2	2
57	1	2	2	12	1						2	2
55	1	1				2				6	2	12
50								9				2
47			2		2	2				9	2	6
42	1		6		2	2				9		
40					2	2		9				2
37								3			2	2
35					1							
32												
25			6									2

Table 4 Full sequence of cloned *S.sobrinus* gene

```
5'      10                  30                  50
GCTCCTCTTG TGACATGGTC ATAGTAACAG ATAATCTGTT TAATTTCAAG CAGATTTAAT

        70                  90                  110
AGCCTCCAGG AAACTTGAAA TAAAACTGAA ATAAAACTGA ATTTTTTATA AAGCCTAGAT

       130                 150                 170
TAAGCAATCG TTTGCATTGA CAATCACTAG ATAAGTGTTA TTATAGATAG TATTGTAACG

       190                 210                 230
AAACATTTCA GATGTTACAA AAATGTAAAT TGGAGGGAAT TATAATATGC AACGAAAAGA
                                                M   Q   R   K   E

       250                 270                 290
GACTTTTGGG TTTCGCAAAA GTAAAATCAG TAGGACCCTT TGTGGTGCCT TACTAGGAAC
  T   F   G   F   R   K   S   K   I   S   R   T   L   C   G   A   L   L   G   T

       310                 330                 350
TGCTATCTTA GCGTCTGTAA CAGGTCAAAA GGCGCTCGCT GAAGAAACAA GTACCACTTC
  A   I   L   A   S   V   T   G   Q   K   A   L   A   E   E   T   S   T   T   S

       370                 390                 410
AACTTCGGGG GTTAATACCG CAGTCGTTGG GACGGAGACT GGGAATCCCG CCACCAACCT
  T   S   G   V   N   T   A   V   V   G   T   E   T   G   N   P   A   T   N   L

       430                 450                 470
GCCTGACAAA CAGGACAATC CAAGTTCGCA AGCCGAGACA AGTCAGGCCC AAGCCGGTCA
  P   D   K   Q   D   N   P   S   S   Q   A   E   T   S   Q   A   Q   A   G   Q

       490                 510                 530
AAAGACAGGG GCAATGTCAG TAGATGTGTC TACAAGTGAG CTTGACGAAG CTGCTAAAAG
  K   T   G   A   M   S   V   D   V   S   T   S   E   L   D   E   A   A   K   S

       550                 570                 590
TGCCCAAGAA GCTGGTGTGA CCGTTTCGCA GGATGCTACC GTCGATAAAG GGACAGTAGA
  A   Q   E   A   G   V   T   V   S   Q   D   A   T   V   D   K   G   T   V   E

       610                 630                 650
AACTTCTGAC GAAGCTAACC AAAAAGAAAC CGAAATCAAG GATGACTACA GCAAGCAAGC
  T   S   D   E   A   N   Q   K   E   T   E   I   K   D   D   Y   S   K   Q   A

       670                 690                 710
AGCAGACATC CAAAAGACAA CAGAAGACTA CAAGGCAGCT GTGGCTCGTA ACCAAGCCGA
  A   D   I   Q   K   T   T   E   D   Y   K   A   A   V   A   R   N   Q   A   E

       730                 750                 770
AACAGACCGA ATCACTCAAG AAAACGCGGC TAAGAAGGCC CAATACGAAC AAGATTTGGC
  T   D   R   I   T   Q   E   N   A   A   K   K   A   Q   Y   E   Q   D   L   A
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      790                   810                   830
GGCCAACAAG GCAGAAGTGG AACGCATTAC CAATGAGAAT GCGCAACGCA AGGCTGATTA
  A  N  K   A  E  V   E  R  I  T   N  E  N   A  Q  R   K  A  D  Y

      850                   870                   890
CGAAGCTAAG CTGGCTCAAT ATCAAAAGGA CCTAGCAGCC GTTCAACAAG CTAATAATGA
  E  A  K   L  A  Q   Y  Q  K  D   L  A  A   V  Q  Q  {A  N  N  D
                                                      A1
      910                   930                   950
CAGTCAAGCA GCCTACGCTG CTGCCAAGGA AGCCTACGAC AAAGAATTGG CTCGGGTTCA
  S  Q  A   A  Y  A   A  A  K  E   A  Y  D   K  E  L   A  R  V  Q

      970                   990                  1010
AGCTGCTAAT GCCGCTGCTA AGAAAGAATA CGAAGAGGCT CTAGCTGCCA ACACCACTAA
  A  A  N   A  A  A   K  K  E  Y   E  E  A   L  A  A   N  T  T  K

     1030                  1050                  1070
GAATGAGCAA ATCAAGGCAG AAAACGCCGC TATCCAGCAA CGCAATGCCC AAGCTAAGGC
  N  E  Q   I  K  A   E  N  A  A   I  Q  Q   R  N  A   Q  A  K  A

     1090                  1110                  1130
AGATTACGAA GCCAAGTTGG CTCAATATGA AAAAGATTTA GCCGCAGCCC AGTCTGGTAA
  D  Y  E   A  K  L   A  Q  Y  E   K  D  L   A  A  A   Q  S}{G  N
                                                          A2
     1150                  1170                  1190
TGCTACAAAT GAAGCGGACT ACCAAGCTAA GAAGGCAGCT TATGAACAAG AGTTAGCGCG
  A  T  N   E  A  D   Y  Q  A  K   K  A  A   Y  E  Q   E  L  A  R

     1210                  1230                  1250
CGTGCAAGCC GCTAATGCAG CTGCCAAGCA GGCCTACGAA CAAGCTCTAG CTGCCAACAC
  V  Q  A   A  N  A   A  A  K  Q   A  Y  E   Q  A  L   A  A  N  T

     1270                  1290                  1310
GGCCAAGAAC GCCCAAATCA CGGCCGAAAA TGAGGCTATC CAGCAGCGCA ATGCGCAAGC
  A  K  N   A  Q  I   T  A  E  N   E  A  I   Q  Q  R   N  A  Q  A

     1330                  1350                  1370
TAAGGCTAAC TATGAAGCTA AATTAGCCCA ATATCAAAAG GATTTGGCCG CAGCTCAATC
  K  A  N   Y  E  A   K  L  A  Q   Y  Q  K   D  L  A   A  A  Q  S}

     1390                  1410                  1430
TGGTAACGCC GCTAATGAGG CAGACTACCA AGAAAAATTA GCAGCCTATG AAAAGGAACT
 {G  N  A   A  N  E   A  D  Y  Q   E  K  L   A  A  Y   E  K  E  L
  A3

     1450                  1470                  1490
GGCTCGTGTG CAAGCAGCCA ATGCAGCTGC TAAGCAAGAA TATGAGCAGA AAGTTCAGGA
  A  R  V   Q  A  A   N  A  A  A   K  Q  E   Y  E  Q   K  V  Q  E

     1510                  1530                  1550
AGCTAATGCT AAAAATGCCG AAATTACGGA AGCCAACCGT GCTATCCGTG AACGCAATGC
  A  N  A   K  N  A   E  I  T  E   A  N  R   A  I  R   E  R  N  A
```

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     1570                1590                1610
CAAGGCCAAG ACAGACTATG AACTCAAACT GTCTAAGTAC CAAGAAGAGC TTGCTCAGTA
  K  A  K   T  D  Y   E  L  K  L   S  K  Y   Q  E  E   L  A  Q  Y

     1630                1650                1670
CAAGAAGGAC CTAGCGGAAT ACCCAGCTAA ACTCCAAGCC TATCAAGATG AACAAGCCGC
  K  K  D   L  A  E   Y  P  A  K   L  Q  A   Y  Q  D   E  Q  A  A

     1690                1710                1730
AATCAAGGCA GCTCTGGAAG AGTTGGAAAA GCACAAGAAT GAAGATTGGA ACCTCAGTGA
  I  K  A   A  L  E   E  L  E  K   H  K  N   E  D  W   N  L  S  E

     1750                1770                1790
GCCCTCAGCC CAGAGTCTGG TCTATGACTT GGAGCCCAAT GCTCAGATTT CCCTAGTGAC
  P  S  A   Q  S  L   V  Y  D  L   E  P  N   A  Q  I   S  L  V  T

     1810                1830                1850
CGATTGGAAG CTACTGAAAG CCTCCTCCCT TGATGAATCC TTTAGCCACG ATACTGAACA
  D  W  K   L  L  K   A  S  S  L   D  E  S   F  S  H   D  T  E  Q

     1870                1890                1910
ATATAACAAA CACAACCTGC AGCCAGATAA TCTAAATATA ACCTATCTGG AGCAGGCTGA
  Y  N  K   H  N  L   Q  P  D  N   L  N  I   T  Y  L   E  Q  A  D

     1930                1950                1970
TGATGTGGCC TCCTCAGTAG AGCTCTTTGG TAATTTCGGT GATAAGGCTG GTTGGACAAC
  D  V  A   S  S  V   E  L  F  G   N  F  G   D  K  A   G  W  T  T

     1990                2010                2030
CACTGTCAGC AATGGTTCAG AAGTTAAGTT TGCCTCTGTC CTCCTCAAGC GTGGCCAGAG
  T  V  S   N  G  S   E  V  K  F   A  S  V   L  L  K   R  G  Q  S

     2050                2070                2090
TGCTACAGCC ACCTATACCA ACCTGAAAAA CTCTTACTAC AATGGTAAGA AAATTTCTAA
  A  T  A   T  Y  T   N  L  K  N   S  Y  Y   N  G  K   K  I  S  K

     2110                2130                2150
GGTGGTCTAC AAGTATACGG TTGACCCTGA CTCCAAGTTT CAAAATCCTA CTGGTAACGT
  V  V  Y   K  Y  T   V  D  P  D   S  K  F   Q  N  P   T  G  N  V

     2170                2190                2210
TTGGTTAGGT ATCTTTACTG ACCCAACCCT AGGGGTCTTT GCCTCAGCCT ATACGGGTCA
  W  L  G   I  F  T   D  P  T  L   G  V  F   A  S  A   Y  T  G  Q

     2230                2250                2270
AAACGAGAAG GATACCTCTA TCTTTATCAA GAATGAATTC ACCTTCTACG ATGAAGACGG
  N  E  K   D  T  S   I  F  I  K   N  E  F   T  F  Y   D  E  D  G

     2290                2310                2330
TAATCCCATC GACTTTGATA ATGCCCTCTT GTCAGTTGCC TCCCTTAACA GGGAACACAA
  N  P  I   D  F  D   N  A  L  L   S  V  A   S  L  N   R  E  H  N
```

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     2350                2370                2390
TTCCATTGAG ATGGCCAAGG ACTACAGCGG TACCTTCGTT AAGATTTCTG GCTCATCCAT
  S  I  E  M  A  K  D  Y  S  G  T  F  V  K  I  S  G  S  S  I

     2410                2430                2450
TGGTGAAAAA AATGGCATGA TCTATGCGAC CGACACCCTC AACTTTAAAA AGGGTGAAGG
  G  E  K  N  G  M  I  Y  A  T  D  T  L  N  F  K  K  G  E  G

     2470                2490                2510
CGGTTCCCTT CACACCATGT ACACCAGAGC AAGTGAGCCT GGTTCAGGTT GGGACTCTGC
  G  S  L  H  T  M  Y  T  R  A  S  E  P  G  S  G  W  D  S  A

     2530                2550                2570
TGATGCTCCT AATTCTTGGT ATGGTGCTGG TGCTGTCAGA ATGTCCGGCC CAAACAACTA
  D  A  P  N  S  W  Y  G  A  G  A  V  R  M  S  G  P  N  N  Y

     2590                2610                2630
CATCACTTTG GGGGCAACCT CAGCGACCAA TGTCCTCAGC CTAGCTGAAA TGCCACAGGT
  I  T  L  G  A  T  S  A  T  N  V  L  S  L  A  E  M  P  Q  V

     2650                2670                2690
ACCTGGTAAA GATAATACTG CTGGTAAAAA ACCAAATATC TGGTATTCCC TTAATGGTAA
  P  G  K  D  N  T  A  G  K  K  P  N  I  W  Y  S  L  N  G  K

     2710                2730                2750
GATTCGGGCA GTCAATGTCC CTAAAGTGAC CAAGGAAAAA CCAACCCCAC CAGTTGAGCC
  I  R  A  V  N  V  P  K  V  T  K  E  K  P  T  P  P  V  E  P

     2770                2790                2810
AACCAAGCCA GACGAGCCAG TCTATGAAGT TGAGAAGGAA TTGGTAGATC TGCCAGTTGA
  T  K  P  D  E  P  V  Y  E  V  E  K  E  L  V  D  L  P  V  E

     2830                2850                2870
ACCAAGCTAC GAAAAGGAAC CAACCCCACC AAGCAAGACT CCAGACCAAA ATATCCCAGA
  P  S  Y  E  K {E  P  T  P  P  S  K  T  P  D  Q  N  I  P  D
                P1

     2890                2910                2930
CAAACCAGTA GAGCCTACTT ATGAGGTTGA AAAGGAGCTG GAACCGGCAC CAGTTGAGCC
  K  P  V  E  P  T  Y  E  V  E  K  E  L  E  P  A  P  V  E  P

     2950                2970                2990
AAGCTACGAA AAGGAACCAA CGCCACCAAG CAAGACTCCG GATCAAGCGA TTCCAGACAA
  S  Y  E  K}{E  P  T  P  P  S  K  T  P  D  Q  A  I  P  D  K
             P2

     3010                3030                3500
ACCGGTAGAG CCAACCTATG AGGTTGAAAA GGAGTTGGAA CCAGTACCTG TAGAAACAAA
  P  V  E  P  T  Y  E__V  E  K  E  L  E  P  V  P  V  E  T  N
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      3070                 3090                 3110
CTACGAAAAG GAACCAACCC CGCCTCAGTC AACCCCAGAC CAAGAAGAGC CCACCAAACC
  Y  E  K} {E  P  T   P  P  Q  S   T  P  D   Q  E  E   P  T  K  P
           P3

      3130                 3150                 3170
GGTGGAACCA AGCTACCAAA GCTTGCCAAC CCCACCAGTG GCACCGACTT ATGAAAAGGT
  V  E  P   S  Y  Q} S  L  P  T   P  P  V   A  P  T   Y  E  K  V

      3190                 3210                 3230
TCCTGGTCCT GTCAGTGTGC CAACGGTTCG GTACCACTAC TATAAACTAG CGGTCCAACC
  P  G  P   V  S  V   P  T  V  R   Y  H  Y   Y  K  L   A  V  Q  P

      3250                 3270                 3290
CGGCGTCACC AAGAAAATCA AAAACCAGGA TGACCTGGAT ATTGACAAGA CCCTGGTGGC
  G  V  T   K  K  I   K  N  Q  D   D  L  D   I  D  K   T  L  V  A

      3310                 3330                 3350
TAAGCAGTCG ACGGTTAAGT TCCAATTGAA GACAGCAGAC CTGCCAGCCG GTCGTCCAGA
  K  Q  S   T  V  K   F  Q  L  K   T  A  D   L  P  A   G  R  P  E

      3370                 3390                 3410
AACGACCTCC TTTGTCTTGA TGGATCCTCT GCCAAGCGGT TACCAACTTA ATCTGGAAGC
  T  T  S   F  V  L   M  D  P  L   P  S  G   Y  Q  L   N  L  E  A

      3430                 3450                 3470
TACCAAGGTC GCCAGCCCAG GCTTTGAAGC TAGCTATGAT GCCATGACCC ATACGGTAAC
  T  K  V   A  S  P   G  F  E  A   S  Y  D   A  M  T   H  T  V  T

      3490                 3510                 3530
CTTCATCGCA ACCGCTGAGA CCTTGGCGGC GCTCAACCAG GATCTGACCA AGGCCGTGGC
  F  I  A   T  A  E   T  L  A  A   L  N  Q   D  L  T   K  A  V  A

      3550                 3570                 3590
GACTATCTAC CCAACAGTTG TGGGACAAGT CCTCAACGAT GGCGCTACCT ACACCAATAA
  T  I  Y   P  T  V   V  G  Q  V   L  N  D   G  A  T   Y  T  N  N

      3610                 3630                 3650
CTTCACCCTG ATGGTCAATG ATGCTTACGG TATTAAATCC AATATCGTTC GCGTGACCAC
  F  T  L   M  V  N   D  A  Y  G   I  K  S   N  I  V   R  V  T  T

      3670                 3690                 3710
ACCAGGGAAA CCTAACGACC CAGACAACCC AAGCAACAAC TACATCACCC CGCACAAGGT
  P  G  K   P  N  D   P  D  N  P   S  N  N   Y  I  T   P  H  K  V

      3730                 3750                 3770
CAAVAAGAAT GAAAACGGTG TGGTGATTGA TGGTAAGTCC GTCCTAGCTG GTACCACCAA
  N  K  N   E  N  G   V  V  I  D   G  K  S   V  L  A   G  T  T  N

      3790                 3810                 3830
CTACTATGAA TTGACTTGGG ACCTGGACCA ATACAAGGGC GATAAATCGG CCAAGGAGAC
  Y  Y  E   L  T  W   D  L  D  Q   Y  K  G   D  K  S   A  K  E  T
```

```
      3850                 3870                 3890
CATCCAAAAA GGCTTCTTCT ATGTGGATGA CTATCCTGAA GAAGCGCTGG ACTTGCGCAC
  I  Q  K   G  F  F   Y  V  D  D   Y  P  E   E  A  L   D  L  R  T

      3910                 3930                 3950
CGACCTGATT AAGCTGACCG ATGCCAACGG CAAGGCGGTC ACTGGTGTCA GCGTGGCTGA
  D  L  I   K  L  T   D  A  N  G   K  A  V   A  G  V   S  V  A  D

      3970                 3990                 4010
CTACGCCAGT CTGGAGGCCG CACCAGCAGC TGTTCAAGAC ATGCTCAAGA AGGCCAACAT
  Y  A  S   L  E  A   A  P  A  A   V  Q  D   M  L  K   K  A  N  I

      4030                 4050                 4070
TACCCCTAAG GGAGCCTTCC AAGTCTTTAC CGCTGACGAT CCTCAGGCCT TCTACGATGC
  T  P  K   G  A  F   Q  V  F  T   A  D  D   P  Q  A   F  Y  D  A

      4090                 4110                 4130
CTATGTGGTT ACCGGAACTG ACCTGACCAT CGTCACTCCA ATGACGGTCA AGGCTGAGAT
  Y  V  V   T  G  T   D  L  T  I   V  T  P   M  T  V   K  A  E  M

      4150                 4170                 4190
GGGCAAGATC GGTGGTAGCT ATGAAAACAA GGCCTACCAG ATTGACTTTG GTAATGGCTA
  G  K  I   G  G  S   Y  E  N  K   A  Y  Q   I  D  F   G  N  G  Y

      4210                 4230                 4250
TGAATCTAAT ATTGTGATTA ACAATGTGCC GCAAATCAAT CCTGAAAAGG ATGTGACCTT
  E  S  N   I  V  I   N  N  V  P   Q  I  N   P  E  K   D  V  T  L

      4270                 4290                 4310
GACCATGGAT CCAGCGGATA GTACCAATGT GGATGGACAG ACCATCGCCC TCAATCAGGT
  T  M  D   P  A  D   S  T  N  V   D  G  Q   T  I  A   L  N  Q  V

      4330                 4350                 4370
CTTTAACTAC CGTCTCATCG GTGGTATCAT TCCAGCGGAC CATGCCGAAG AGCTCTTTGA
  F  N  Y   R  L  I   G  G  I  I   P  A  D   H  A  E   E  L  F  E

      4390                 4410                 4430
GTACAGCTTT AGCGATGACT ATGACCAAAC TGGAGACCAG TACACGGGCC AATACAAGGC
  Y  S  F   S  D  D   Y  D  Q  T   G  D  Q   Y  T  G   Q  Y  K  A

      4450                 4470                 4490
CTTTGCCAAG GTTGACCTGA CCCTCAAGGA TGGTACAATC ATCAAGGCGG GTACTGACTT
  F  A  K   V  D  L   T  L  K  D   G  T  I   I  K  A   G  T  D  L

      4510                 4530                 4550
GACTTCATAT ACAGAAGCGC AAGTTGATGA AGCTAATGGC CAAATTGTTG TGACCTTCAA
  T  S  Y   T  E  A   Q  V  D  E   A  N  G   Q  I  V   V  T  F  K

      4570                 4590                 4610
GGAAGATTTC TTGCGGTCTG TGTCTGTAGA CTCGGCCTTC CAAGCGGAAG TCTACCTACA
  E  D  F   L  R  S   V  S  V  D   S  A  F   Q  A  E   V  Y  L  Q
```

```
      4630                  4650                  4670
GATGAAGCGG ATAGCCGTCG GGACCTTTGC CAATACCTAT GTCAATACGG TCAATGGAAT
  M  K  R   I  A  V   G  T  F  A   N  T  Y   V  N  T   V  N  G  I

      4690                  4710                  4730
TACCTATAGC TCTAATACGG TAAGGACCAG CACACCAGAG CCGAAGCAGC CAAGTCCAGT
  Y  Y  S   S  N  T   V  R  T  S   T  P  E   P  K  Q   P  S  P  V

      4750                  4770                  4790
GGTACCTAAG ACCACTACTA CGGTAGTCTT CCAGCCTCGT CAGGGTCAAG CTTATCAGCC
  V  P  K   T  T  T   T  V  V  F   Q  P  R   Q  G  Q   A  Y  Q  P

      4810                  4830                  4850
AGCGCCGCCA GCAGGAGCTC AATTGCCAGC CACAGGGGAT AGTAGCAATG CTTACCTGCC
  A  P  P   A  G  A   Q  L  P  A   T  G  D   S  S  N   A  Y  L  P
                        -.-.-.- .-.-.-

      4870                  4890                  4910
ACTTTTAGGC CTCGTAAGCC TGACTGCTGG CTTTAGCCTG TTAGGACTGC GCCGGAAGCA
  L  L  G   L  V  S   L  T  A  G   F  S  L   L  G  L   R  R  K  Q

      4930                  4950                  4970
GGACTAAAGA ATCCAACAAG AAAAAATGGG AAAGTTTGCC TTTCTCATTT TTTATATTCC
  D  *

      4990                  5010                  5030
CAGCTAGCTG AGTAGTCAAG AAGTACTCTT AGAAAACCCT AGAGAACATT AGCTAACTTT

      5050                  5070
TCCAAACCGA TAGACGTTTA TTTTAGTCTA AGTATGG
```

Table 5 The *S.oralis* DNA Sequence Encoding an Endocarditis - Specific Antigen Region

```
5'      10                  30                  50
GAATTCACCT TCTACGATGA AAATGACCAA CCAATTAATT TTGACAATGC TCTTCTTTCA
 E  F  T   F  Y  D  E   N  D  Q   P  I  N   F  D  N  A   L  L  S

        70                  90                  110
GTAGCCTCAC TTAACCGTGA GCATAACTCT ATTGAGATGG CTAAGGATTA TAGTGGTACT
 V  A  S   L  N  R  E   H  N  S   I  E  M   A  K  D  Y   S  G  T

       130                 150                 170
TTTATTAAAA TCTCAGGTTC ATCCATCGGT GAAAAAAATG GCATGATTTA TGCCACAGAA
 F  I  K   I  S  G  S   S  I  G   E  K  N   G  M  I  Y   A  T  E

       190                 210                 230
ACCCTGAACT TTAAACAAGG ACAGGGTGGA GCTCGCTGGA CAATGTATCC AAATCGTCAG
 T  L  N   F  K  Q  G   Q  G  G   A  R  W   T  M  Y  P   N  R  Q

       250                 270                 290
CCAGGTTCAG GTTGGGATTC ATCAGATGCA CCAAACTCTT GGTACGGTGC AGGGGCCATT
 P  G  S   G  W  D  S   S  D  A   P  N  S   W  Y  G  A   G  A  I

       310                 330                 350
AGTATGTCCG GTCCTACGAA TCACGTTACA GTTGGTGCAA CATCTGCTAC CAATGTGATG
 S  M  S   G  P  T  N   H  V  T   V  G  A   T  S  A  T   N  V  M

       370                 390                 410
TCCGTAGCAG AAATGCCTCA AGTACCTGGA AGAGACAATA CTGAAGGTAA AAGACCAAAC
 S  V  A   E  M  P  Q   V  P  G   R  D  N   T  E  G  K   R  P  N

       430                 450                 470
ATCTGGTACT CACTCAATGG TAAAATTCGT GCGGTTGACG TTCCGAAAAT TACAAAAGAA
 I  W  Y   S  L  N  G   K  I  R   A  V  D   V  P  K  I   T  K  E

       490                 510                 530
AAACCAACTC CACCGGTAGC ACCAACTGAA CCACAAGCTC CTACCTATGA AGTGGAGAAA
 K  P  T   P  P  V  A   P  T  E   P  Q  A   P  T  Y  E   V  E  K

       550                 570                 590
CCACTGGAAC CGGCTCCAGT AGCACCAAGC TACGAAAATG AGCCAACTCC ACCAGTAAAA
 P  L  E   P  A  P  V   A  P  S   Y  E  N  {E  P  T  P   P  V  K
                                              P1

       610                 630                 650
ACTCCAGATC AACCGGAGCC ATCAAAACCA GAAGAGCCAA CATATGAGAC AGAGAAACCA
 T  P  D   Q  P  E  P   S  K  P   E  E  P   T  Y  E  T   E  K  P
```

```
       670                  690                  710
TTGGAACCAG CTCCAGTAGC ACCAAACTAC GAAAATGAGC CAACTCCACC AGTAAAAACT
 L  E  P   A  P  V  A   P  N  Y   E  N}{E   P  T  P  P   V  K  T
                                        P2

       730                  750                  770
CCAGATCAAC CAGACCCATC AAAACCGGAA GAGCCAAACT ATGAGACAGA GAAACCATTG
 P  D  Q   P  D  P  S   K  P  E   E  P  N   Y  E  T  E   K  P  L

       790                  810                  830
GAACCAGCTC CAGTAGCACC AAGCTATGAA AATGAGCCAA CTCCACCGGT AAAAACTCCA
 E  P  A   P  V  A  P   S  Y  E   N}{E  P   T  P  P  V   K  T  P
                                        P3

       850                  870                  890
GATCAACCAG AGCCATCAAA ACCAGAAGAG CCAAATTATG ATCCATTGCC AACTCCGCCG
 D  Q  P   E  P  S  K   P  E  E   P  N  Y   D} P  L  P   T  P  P

       910                  930                  950
CTAGCACCAA CTCCTAAGCA GTTGCCAACA CCACCAGCGG TGCCAACAGT TCACTTCCAT
 L  A  P   T  P  K  Q   L  P  T   P  P  A   V  P  T  V   H  F  H

       970                  990                 1010
TACAATCGTC TATTTGCACA ACCTCAGATT AATAAAGAAA TTAAAAACGA GGATGGAGTA
 Y  N  R   L  F  A  Q   P  Q  I   N  K  E   I  K  N  E   D  G  V

      1030                 1050                 1070
GATATTGATC GTACTCTAGT TGCTAAGCAG TCTGTAGTGA AGTTTGAGCT GAAAACAGAA
 D  I  D   R  T  L  V   A  K  Q   S  V  V   K  F  E  L   K  T  E

      1090                 1110                 1130
GCTTTAACTG CTGGTCGTCC AAAAACAACT TCGTTTGTAT TGGTAGATCC ACTTCCAACT
 A  L  T   A  G  R  P   K  T  T   S  F  V   L  V  D  P   L  P  T

      1150                 1170                 1190
GGCTATCAGT TTGATTTGGA AGCAACCAAG GCTGCAAGCA AAGGTTTTGA AACAAGCTAT
 G  Y  Q   F  D  L  E   A  T  K   A  A  S   K  G  F  E   T  S  Y

      1210                 1230                 1250
GACAAAGCTA GTCACACTGT AACCTTTAAG GCTACTGAGG AGACCTTAGC TGCTTTCAAT
 D  K  A   S  H  T  V   T  F  K   A  T  E   E  T  L  A   A  F  N

      1270                 1290                 1310
GCTGATTTGA CAAAATCCTT TGAGACTCTA TATCCAACTG TTGTTGGTCG TGTCTTGAAT
 A  D  L   T  K  S  F   E  T  L   Y  P  T   V  V  G  R   V  L  N

      1330                 1350                 1370
GATGGGGCGA CTTATACGAA TAACTTTACA TTGACAGTCA ACGATGCTAC TGGTGTCAAG
 D  G  A   T  Y  T  N   N  F  T   L  T  V   N  D  A  T   G  V  K
```

```
      1390                 1410                 1430
TCAAACATTG TTCGTGTAAC GACTCCAGGT AAACCAAATG ATCCTGACAA TCCAAATAAC
 S  N  I   V  R  V  T   T  P  G   K  P  N   D  P  D  N   P  N  N

      1450                 1470                 1490
AACTACATCA AGCCTTTGAA AGTTAACAAG AACAAGCAAG GTGTGAATAT TGATGGCAAA
 N  Y  I   K  P  L  K   V  N  K   N  K  Q   G  V  N  I   D  G  K


      1510                 1530                 1550
GAAGTTCTAG CTGGTTCAAC GAACTACTAT GAACTCACAT GGGATTTGGA TCAATACAAG
 E  V  L   A  G  S  T   N  Y  Y   E  L  T   W  D  L  D   Q  Y  K

      1570                 1590                 1610
GGAGATAAAT CTTCTAAAGA AGCGATTCAA AATGGTTTCT ACTATGTGGA TGATTATCCA
 G  D  K   S  S  K  E   A  I  Q   N  G  F   Y  Y  V  D   D  Y  P

      1630                 1650                 1670
GAAGAAGCTT TAACGCTTCA ACCAGAATTG GTTAAGATTC GTGATCTAGA GGGCAACCTT
 E  E  A   L  T  L  Q   P  E  L   V  K  I   R  D  L  E   G  N  L

      1690                 1710                 1730
GTATCAGGTA TCAGTGTTCA ACAGTTTGAT AGTTTAGAAC GTGCGCCTAA GAAGGTTCAA
 V  S  G   I  S  V  Q   Q  F  D   S  L  E   R  A  P  K   K  V  Q

      1750                 1770                 1790
GATCTGTTGA AGAAAGCAAA CATCACTGTT AAAGGTGCTT TCCAACTCTT CTCAGCTGAT
 D  L  L   K  K  A  N   I  T  V   K  G  A   F  Q  L  F   S  A  D

      1810
AATCCAGCTG AATTC
 N  P  A   E  F
```

This 1.81 kb DNA fragment carried the three tandem repeat proline (P1 - 3) common to the family of antigen 1/11 proteins important in dental caries. The repeat proline rich region of this amino acid sequence was bracketed ({}) as above.

Table 6

Peptide Number	Amino Acid Sequence
1	YEVEKPLEPAPVAPS
2	SYENEPTPPVKTPDC
3	KTPDQPEPSKPEEPT
4	EPAPVAPSYENEPTP
5	YEVEKELVDLPVEPS
6	KTPDQNIPDKPVEPT

Table 7

Clinical History of Serum

Peptide Number

	1		2		3		4		5		6	
	M	G	M	G	M	G	M	G	M	G	M	G
S.mutans endocarditis	0.341	1.506	0.331	0.381	0.338	1.365	0.445	1.225	0.261	1.491	0.241	0.869
S.oralis endocarditis	0.442	0.902	0.561	0.891	0.491	0.768	0.567	0.936	0.653	0.784	0.373	0.873
S.oralis septicaemia	0.024	0.118	0.036	0.215	0.019	0.123	0.065	0.168	0.043	0.241	0.117	0.218
S.lactis endocarditis	0.306	0.589	0.319	0.528	0.309	0.492	0.377	0.415	0.337	0.421	0.244	0.449
S.aureus endocarditis	0.075	0.177	0.145	0.579	0.068	0.153	0.015	0.127	0.101	0.267	0.063	0.217

ELISA readings for peptides 1-6

Table 8

Indirect ELISA results for Peptide 1

Concentration of peptide: 200µl at 10µg/ml
Primary antibody 1/100

Patient No.	Sera Date	Mean[a] Blank O.D.	Mean Sample O.D.[b]		Standard Deviation	
			IgM	IgG	IgM	IgG
S.mutans Endocarditis						
1	16.03.88	0.131	0.117	0.698	0.001	0.015
2	17.09.87	0.193	0.012	0.742	0.003	0.0017
S.oralis Endocarditis (n=3)						
3	23.05.78	0.141	0.462	0.960	0.018	0.002
4	12.12.81	0.197	0.447	0.902	0.023	0.031
5	23.02.84 30.08.84	0.121 0.131	0.043 0.153	0.038 0.699	0.001 0.004	0.008 0.007
S.gordonii Endocarditis (n=14)						
6	1.07.86 15.08.86 22.09.86 6.10.96	0.131 0.131 0.131 0.131	0.422 0.339 0.228 0.173	0.936 0.855 0.81 1.06	0.001 0.003 0.010 0.003	0.002 0.004 0.005 0.002
7	8.01.87	0.131	0.487	0.685	0.010	0.012
8	18.12.86 21.01.87	0.131 0.131	0.059 0.270	0.662 0.715	0.004 0.005	0.006 0.009
9	10.08.86	0.131	0.43	0.41	0.007	0.004
10	17.07.86	0.131	0.568	0.601	0.010	0.012
11	14.07.93	0.131	0.095	0.471	0.012	0.003
12	20.03.93 22.03.93	0.158 0.158	0.164 0.168	0.404 0.475	0.001 0.003	0.03 0.03
13	06.05.87 12.08.87	0.121 0.121	0.438 0.274	0.079 0.579	0.023 0.021	0.003 0.016

14	7.06.86 9.06.86 16.06.86 19.06.86 23.06.86 26.06.86	0.131 0.151 0.153 0.193 0.151 0.153	0.174 0.144 0.151 0.134 0.149 0.106	0.459 0.469 0.587 0.706 0.562 0.475	0.006 0.004 0.005 0.006 0.007 0.005	0.024 0.013 0.011 0.008 0.011 0.017
15	2.12.87 30.12.87	0.012 0.131	0.301 0.217	1.391 1.083	0.006 0.005	0.011 0.007
S.sanguis Endocarditis (n=2)						
16	21.07.86 7.08.86	0.121 0.121	0.46 0.487	0.543 0.685	0.006 0.001	0.011 0.011
17	14.07.93	0.121	0.416	0.401	0.049	0.022
S.oralis Septicaemics (n=8)						
18	23.11.88 6.12.88	0.133 0.131	0.046 0.024	0.112 0.118	0.017 0.001	0.001 0.004
19	22.08.89 6.09.89 17.10.89	0.131 0.144 0.121	0.023 0.034 0.006	0.194 0.205 0.042	0.001 0.0016 0.003	0.003 0.001 0.004
20	11.03.88 1.04.88 10.05.88	0.197 0.133 0.197	0.007 0.043 0.021	0.048 0.102 0.014	0.018 0.003 0.001	0.008 0.011 0.001
21	27.06.89 1.80.89	0.197 0.151	0.058 0.003	0.031 0.095	0.015 0.002	0.021 0.001
22	2.08.88	0.144	0.017	0.082	0.001	0.014
23	4.04.89	0.135	0.044	0.061	0.002	0.002
24	22.03.93 22.04.93 26.04.93 27.04.93	0.193 0.158 0.139 0.158	0.029 0.032 0.034 0.042	0.022 0.041 0.044 0.036	0.001 0.003 0.011 0.001	0.001 0.001 0.021 0.009
25	21.07.93 21.07.93 4.08.93 17.08.93	0.139 0.139 0.139 0.139	0.089 0.113 0.129 0.095	0.027 0.031 0.032 0.103	0.003 0.009 0.001 0.003	0.009 0.005 0.008 0.019
E.faecalis Endocarditis						

26	25.02.93 25.02.93 29.02.93	0.141 0.141 0.141	0.027 0.024 0.026	0.075 0.063 0.071	0.001 0.001 0.003	0.014 0.003 0.001
27	15.06.93 16.06.93	0.147 0.147	0.085 0.071	0.248 0.275	0.003 0.002	0.007 0.013
28	20.01.92	0.141	0.041	0.069	0.004	0.001
29	24.06.88	0.153	0.085	0.283	0.003	0.017
30	10.06.86	0.135	0.054	0.361	0.003	0.012
31	19.11.87	0.133	0.033	0.652	0.009	0.006
32	29.01.89 13.10.89 16.11.89 8.01.90	0.131 0.133 0.197 0.133	0.081 0.024 0.016 0.043	0.564 0.281 0.195 0.371	0.011 0.003 0.006 0.008	0.016 0.022 0.005 0.018
33	10.05.88 15.05.88 21.05.88 28.08.88	0.197 0.131 0.131 0.131	0.096 0.088 0.048 0.156	1.079 1.011 0.531 0.674	0.003 0.003 0.002 0.003	0.018 0.018 0.015 0.013
34	6.11.86	0.141	0.086	0.082	0.013	0.001
35	12.01.87	0.144	0.039	0.387	0.008	0.022
36	5.01.87	0.135	0.011	0.559	0.001	0.015
E.faecalis Septicaemics						
37	30.12.87	0.133	0.029	0.371	0.001	0.007
38	10.10.85	0.193	0.018	0.175	0.004	0.025
39	24.08.89 11.10.85 11.10.85	0.193 0.151 0.153	0.033 0.052 0.049	0.002 0.266 0.218	0.003 0.001 0.011	0.002 0.008 0.014
40	8.01.88	0.135	0.041	0.286	0.019	0.001
41	27.11.87	0.197	0.034	0.354	0.001	0.004
42	20.04.85	0.144	0.02	0.132	0.003	0.006
43	27.06.88	0.144	0.034	0.152	0.013	0.011
E. faecium Septicaemics						
44	19.07.85	0.197	0.135	0.436	0.015	0.026

45	23.10.85 28.11.85 2.12.85	0.144 0.139 0.139	0.029 0.027 0.055	0.269 0.061 0.057	0.011 0.001 0.003	0.008 0.001 0.003
S.bovis Endocarditis						
46	8.07.86	0.133	0.155	0.429	0.023	0.013
47	22.03.85 27.03.85	0.193 0.151	0.027 0.012	0.021 0.153	0.002 0.003	0.003 0.019
48	12.03.87 6.05.87 12.08.87	0.139 0.121 0.121	0.255 0.438 0.274	0.124 0.079 0.141	0.001 0.023 0.021	0.007 0.003 0.016
49	30.10.85	0.131	0.073	0.242	0.011	0.022
50	8.05.86	0.153	0.162	0.593	0.001	0.019
51	12.04.85 26.04.85 30.04.85	0.153 0.153 0.193	0.203 0.241 0.025	0.823 1.133 0.501	0.004 0.001 0.002	0.015 0.001 0.011
52	30.12.87	0.144	0.063	0.197	0.003	0.003
53	18.07.93 18.07.93 21.07.93 21.07.93 24.07.93 25.07.93 25.07.93	0.139 0.139 0.139 0.139 0.139 0.139 0.139	0.191 0.177 0.108 0.122 0.135 0.141 0.293	0.069 0.066 0.035 0.038 0.054 0.073 0.051	0.019 0.009 0.011 0.004 0.003 0.013 0.019	0.008 0.004 0.006 0.009 0.008 0.015 0.006
S.agalactiae Endocarditis						
54	20.06.88 22.06.88	0.135 0.135	0.025 0.088	0.104 0.484	0.001 0.019	0.005 0.011
S.lactis Endocarditis						
55	24.03.88	0.135	0.021	0.218	0.011	0.008
S.pneumoniae Endocarditis						
56	4.12.87	0.133	0.095	0.492	0.011	0.019
57	16.07.86	0.131	0.103	0.281	0.008	0.006
58	7.10.86	0.144	0.101	0.591	0.009	0.003
59	21.10.86	0.131	0.033	0.569	0.011	0.008

Group G *Streptococcal* endocarditis						
60	29.10.86	0.131	0.131	0.651	0.009	0.019
61	27.03.85	0.197	0.111	0.789	0.017	0.015
NON NEUTROPENIC CONTROLS *Staphylococcus aureus* Endocarditis						
62	8.02.88	0.149	0.087	0.077	0.029	0.018
63	28.02.88	0.149	0.106	0.037	0.004	0.008
Coagulase Negative *Staphylococcus* Endocarditis						
64	30.09.89	0.139	0.054	0.062	0.007	0.001
65	16.06.89 1.07.86	0.153 0.131	0.051 0.146	0.215 0.472	0.013 0.003	0.007 0.015
66	24.02.87 5.03.87	0.139 0.121	0.243 0.387	0.083 0.098	0.001 0.008	0.025 0.006
67	2.01.88	0.144	0.016	0.192	0.006	0.019
68	10.08.86 25.10.86	0.144 0.139	0.029 0.067	0.287 0.071	0.012 0.008	0.019 0.006
69	15.02.88	0.133	0.075	0.177	0.011	0.008
Endocarditis due to *Candida albicans*						
70	24.10.86	0.147	0.244	0.159	0.004	0.003
Endocarditis due to *Candida parapsilosis*						
71	1.02.88 11.06.88 18.06.88	0.153 0.139 0.158	0.077 0.121 0.258	0.192 0.165 0.216	0.006 0.005 0.007	0.008 0.006 0.004
72	8.05.86	0.139	0.087	0.081	0.008	0.007
Endocarditis due to *Escherichia coli*						
73	1.07.88 2.07.88 5.07.88	0.135 0.133 0.197	0.038 0.022 0.073	0.358 0.379 0.344	0.003 0.011 0.008	0.018 0.011 0.002
SLE						
74	13.06.93 16.06.93	0.158 0.147	0.327 0.322	0.167 0.245	0.004 0.008	0.011 0.022

75	2.05.88	0.144	0.023	0.285	0.009	0.004
76	18.04.89	0.193	0.015	0.304	0.004	0.003
Brain abscess due to *Streptococcus milleri*						
77	17.10.86 30.10.86 6.11.86	0.153 0.151 0.193	0.182 0.342 0.011	0.312 0.353 0.185	0.006 0.011 0.003	0.022 0.001 0.013
NEUTROPENIC CONTROLS						
78	7.08.88	0.193	0.019	0.183	0.004	0.019
79	11.11.86	0.151	0.101	0.251	0.013	0.009
80	21.10.86	0.149	0.101	0.078	0.015	0.018
81	25.07.91	0.139	0.024	0.045	0.003	0.001
82	9.02.89	0.012	0.151	0.147	0.003	0.003
83	15.06.93	0.151	0.021	0.177	0.004	0.009
84	23.06.92	0.193	0.016	0.176	0.025	0.015
85	19.01.88	0.149	0.108	0.033	0.015	0.001
86	25.10.88	0.193	0.007	0.035	0.005	0.004
87	21.03.86	0.158	0.026	0.045	0.006	0.008
88	18.02.86	0.139	0.037	0.081	0.001	0.008
89	6.08.91	0.144	0.034	0.421	0.005	0.014
90	24.04.87	0.133	0.017	0.023	0.003	0.003
91	12.12.89	0.144	0.013	0.047	0.001	0.004
92	30.10.86	0.139	0.076	0.125	0.009	0.014
93	2.07.86	0.139	0.125	0.094	0.007	0.003
94	5.02.87	0.149	0.119	0.032	0.001	0.003
95	5.09.98	0.135	0.017	0.021	0.004	0.003
96	5.08.93	0.153	0.055	0.152	0.014	0.026
97	19.08.93 1.09.93 14.09.93	0.158 0.158 0.158	0.022 0.044 0.026	0.058 0.088 0.144	0.001 0.009 0.003	0.004 0.006 0.022
98	3.05.87	0.141	0.131	0.252	0.004	0.014

a Mean blank O.D., this value was obtained for each microtitre plate by calculation of the mean O.D. for the six wells containing stain only. This mean blank O.D. was subtracted from the each of the sample O.D. measurements to produce the corrected mean sample O.D. value.

b Mean sample O.D., this was the value obtained after subtraction of the mean blank O.D. value from the mean of the three O.D. measurements taken from each of the sample wells.

Table 8a

Indirect ELISA results
Concentration of peptides: 200ml at 10ug/ml
Primary antibody 1/100
[a]Peptide numbers 1,7 and 8 as before.

Patient No	Mean Sample Optical Density					
	IgM			IgG		
	1[a]	7[a]	8[a]	1[a]	7[a]	8[a]
S.mutans Endocarditis						
1	0.117	0.636	0.862	0.698	0.602	0.733
S,oralis Endocarditis						
4	0.447	0.584	1.236	0.902	0.433	0.678
S.gordonii Endocarditis						
6	0.422	0.742	1.443	0.936	0.524	1.694
8	0.27	0.582	0.891	0.715	0.319	1.01
9	0.43	1.103	0.95	0.41	0.388	0.19
10	0.568	0.700	0.824	0.601	0.319	0.505
11	0.095	0.641	1.396	0.471	0.685	0.889
12	0.164	0.988	1.399	0.404	0.828	0.859
13	0.274	1.098	1.605	0.579	0.841	0.799
14	0.174	0.991	1.600	0.459	0.542	0.64
S.sanguis Endocarditis						
16	0.46	1.335	1.366	0.543	1.802	2.267
17	0.417	1.126	1.07	0.401	0.335	0.632
S.oralis Septicaemias						
18	0.046	0.136	0.243	0.112	0.324	0.536
19	0.023	0.324	0.314	0.194	0.294	0.586

20	0.007	0.228	0.295	0.048	0.272	0.607
21	0.058	0.206	0.280	0.031	0.289	0.476
22	0.017	0.316	0.879	0.982	0.384	0.824
24	0.029	0.911	1.327	0.022	0.475	0.754
25	0.089	0.348	0.720	0.027	0.238	0.369
E. faecalis Endocarditis						
26	0.027	0.344	0.390	0.075	0.376	0.636
32	0.081	0.226	0.317	0.564	0.388	0.756
35	0.039	1.133	1.228	0.387	0.906	1.086
36	0.011	0.549	0.926	0.559	0.591	1.176
E.faecalis Septicaemias						
38	0.018	0.784	1.344	0.175	0.39	0.796
39	0.033	0.590	0.78	0.002	0.432	0.87
E.faecium Septicaemias						
45	0.144	0.47	1.228	0.269	0.588	1.086
S.oralis Endocarditis						
51	0.203	1.063	1.075	0.823	0.410	0.802
S,aureus Endocarditis						
62	0.087	0.333	0.266	0.077	0.435	0.401
63	0.106	0.356	0.805	0.037	0.484	0.646
Coagulase negative Staphylococcus endocarditis						
64	0.054	0.596	0.879	0.062	0.612	0.829
68	0.029	1.502	1.205	0.287	1.446	1.521
SLE						
74	0.327	0.697	1.509	0.167	0.542	1.839

Brain abscess *S.milleri*						
77	0.182	1.421	2.162	0.312	0.500	0.781
Neutropenic Controls						
78	0.193	0.570	0.551	0.183	0.804	0.428
79	0.151	0.579	0.491	0.251	0.726	0.428
80	0.149	0.744	0.628	0.078	0.525	0.512
81	0.139	0.422	0.414	0.045	0.530	0.412
82	0.012	0.406	0.382	0.147	0.931	0.809
83	0.151	0.328	0.394	0.177	0.233	0.396
84	0.193	0.601	0.533	0.176	0.950	0.683
85	0.149	0.581	0.34	0.033	0.372	0.60
86	0.193	0.456	0.325	0.035	0.616	0.313
87	0.158	0.610	0.597	0.045	0.542	0.377
88	0.139	0.559	0.475	0.081	0.682	0.497
89	0.144	0.628	0.130	0.421	0.477	0.166
90	0.133	0.142	0.182	0.023	0.280	0.730
91	0.144	0.394	0.305	0.047	0.636	0.317
92	0.139	0.403	0.323	0.125	0.616	0.588
93	0.139	1.021	0.23	0.094	0.858	0.57
94	0.149	0.42	0.47	0.032	0.53	0.432
95	0.135	0.463	0.436	0.021	0.711	0.674
96	0.153	0.568	0.539	0.152	0.760	0.587
97	0.158	0.743	0.650	0.058	0.914	0.535
98	0.141	0.366	0.384	0.252	0.528	0.366

Table 9

	IgG ≥0.6	IgM ≥0.4	IgM ≥0.4 and IgG ≥0.6
S.mutans endocarditis	100%	0%	100%
S.oralis endocarditis	100%	66%	100%
S.gordonii endocardits	60%	50%	80%
S.sanguis endocarditis	50%	100%	100%
S.oralis septicaemias	0%	0%	0%
E.faecalis endocarditis	18%	0%	18%
E.faecalis septicaemia	0%	0%	0%
E.faecium septicaemia	0%	0%	0%
S.bovis endocardits	12.5%	12.5%	25%
S.agalactiae endocarditis	0%	0%	0%
S.lactis endocarditis	0%	0%	0%
S.pneumoniae endocarditis	0%	0%	0%
Group G streptococcal endocarditis	100%	0%	100%
Staphylococcus aureus endocardits	0%	0%	0%
Coagulase Negative endocarditis	0%	0%	0%
Candida albicans endocarditis	0%	0%	0%
Candida parapsilosis endocarditis	0%	0%	0%
E. coli endocarditis	0%	0%	0%
SLE	0%	0%	0%
S.milleri	0%	0%	0%
Neutropenic controls	0%	0%	0%

Table 10

Epitope Number	Epitope Sequence	Negative Control	Viridans Endocarditis	*S. oralis* Septicaemia	*S. mutans* Endocarditis	*E. faecalis* Endocarditis
		n=3	n=8	n=5	n=2	n=2
		O.D. S.D	O.D. S.D.	O.D. S.D.	O.D. S.D.	O.D. S.D.
61	NFKQGQG	0.400 0.027	1.039 0.353	0.429 0.200	0.833 0.125	0.588 0.054
62		0.388 0.034	0.972 0.347	0.419 0.230	0.808 0.122	0.508 0.027
63		0.444 0.058	0.992 0.329	0.430 0.215	0.888 0.195	0.540 0.013
74	RQPG	0.341 0.019	0.902 0.319	0.322 0.211	0.839 0.004	0.407 0.120
75		0.283 0.033	1.038 0.470	0.351 0.180	0.891 0.011	0.450 0.119
76		0.282 0.034	1.053 0.474	0.455 0.180	0.917 0.029	0.493 0.140
77		0.350 0.045	0.981 0.358	0.309 0.070	1.041 0.043	0.472 0.130
78		0.280 0.023	0.918 0.425	0.307 0.080	1.142 0.405	0.423 0.096
79		0.282 0.015	0.781 0.312	0.271 0.091	1.005 0.418	0.373 0.084
90	SWYGAG	0.308 0.054	0.852 0.318	0.287 0.070	1.027 0.192	0.378 0.088
91		0.316 0.069	0.924 0.340	0.328 0.080	1.064 0.138	0.428 0.136
92		0.344 0.076	0.917 0.429	0.298 0.060	0.914 0.033	0.377 0.115
93		0.306 0.007	0.957 0.378	0.444 0.140	1.083 0.225	0.483 0.131
144	GKIRAV	0.306 0.072	0.824 0.318	0.348 0.180	0.779 0.129	0.389 0.115
145		0.384 0.071	1.028 0.309	0.437 0.210	0.971 0.199	0.515 0.120
148		0.303 0.042	0.852 0.261	0.348 0.150	0.881 0.240	0.438 0.115
147		0.395 0.035	0.933 0.285	0.397 0.180	0.944 0.258	0.483 0.131
320	RLFAQPQ	0.392 0.031	1.147 0.657	0.491 0.180	1.010 0.151	0.494 0.115
321		0.453 0.049	1.209 0.417	0.548 0.230	1.284 0.300	0.649 0.171
322		0.467 0.124	1.145 0.356	0.531 0.190	1.289 0.346	0.593 0.116
359	AGRPK	0.395 0.043	0.921 0.295	0.401 0.200	0.749 0.177	0.480 0.040
360		0.492 0.065	1.049 0.374	0.428 0.240	0.775 0.030	0.491 0.062
361		0.407 0.032	0.907 0.318	0.382 0.190	0.657 0.094	0.0462 0.067
362		0.430 0.008	1.054 0.325	0.423 0.230	0.753 0.091	0.525 0.082
363		0.421 0.042	0.946 0.279	0.391 0.021	0.665 0.088	0.477 0.083
376	PTGYQFD	0.277 0.035	0.791 0.479	0.428 0.080	0.762 0.190	0.339 0.095
377		0.415 0.039	1.045 0.427	0.359 0.140	1.548 0.728	0.487 0.155
378		0.351 0.062	0.802 0.290	0.291 0.140	1.274 0.793	0.380 0.151
426	YPTVV	0.309 0.081	0.852 0.391	0.269 0.100	1.275 0.617	0.410 0.147
427		0.215 0.084	0.473 0.161	0.225 0.050	0.525 0.117	0.289 0.030
428		0.325 0.013	1.170 0.457	0.450 0.180	1.143 0.125	0.561 0.194
429		0.339 0.046	0.964 0.336	0.413 0.150	1.050 0.179	0.488 0.165
430		0.349 0.088	0.976 0.328	0.465 0.140	1.002 0.198	0.458 0.139
577	LLKKA	0.279 0.042	0.863 0.302	0.353 0.190	0.681 0.142	0.466 0.081
578		0.349 0.019	0.907 0.260	0.410 0.210	0.808 0.270	0.557 0.103
579		0.351 0.051	0.937 0.254	0.415 0.210	0.882 0.221	0.549 0.098
580		0.395 0.077	1.035 0.267	0.423 0.210	0.898 0.183	0.587 0.124
581		0.331 0.029	0.847 0.261	0.581 0.150	0.843 0.153	0.482 0.141

Table 11

Peptide Number	Viridans Endocarditis	*S.oralis* Septicaemia	*S. mutans* Endocarditis	*E. faecalis* Endocarditis
	n = 8	n = 5	n = 2	n = 2
61	7/8	1/5	2/2	0/2
62	5/8	1/5	2/2	0/2
63	5/8	1/5	2/2	0/2
74	6/8	0/5	2/2	0/2
75	7/8	0/5	2/2	0/2
76	7/8	0/5	2/2	0/2
77	6/8	0/5	2/2	0/2
78	6/8	0/5	2/2	0/2
79	4/8	0/5	2/2	0/2
90	5/8	0/5	2/2	0/2
91	5/8	0/5	2/2	0/2
92	5/8	0/5	2/2	0/2
93	5/8	0/5	2/2	0/2
14	6/8	0/5	1/2	0/2
145	7/8	1/5	2/2	0/2
146	6/8	0/5	2/2	0/2
147	6/8	0/5	2/2	0/2
320	7/8	1/5	2/2	0/2
321	8/8	1/5	2/2	1/2
322	7/8	1/5	2/2	0/2
359	6/8	1/5	1/2	0/2
360	7/8	1/5	2/2	0/2
361	5/8	1/5	1/2	0/2
362	7/8	1/5	1/2	0/2
263	6/8	1/5	1/2	0/2
376	4/8	0/5	1/2	0/2
377	6/8	0/5	2/2	0/2
378	4/8	0/5	2/2	0/2
426	4/8	0/5	2/2	0/2
427	1/8	0/5	0/2	0/2
428	7/8	1/5	2/2	0/2
429	7/8	0/5	2/2	0/2
430	5/8	0/5	2/2	0/2
577	5/8	0/5	1/2	0/2
578	6/8	1/5	1/2	0/2
579	6/8	1/5	2/2	0/2
580	8/8	1/5	2/2	0/2
581	5/8	0/5	2/2	0/2

Positive is > 0.700

CLAIMS

1. A purified bacterial protein expressed during infection due to streptococci or enterococci and isolated from human sera having at least the sequence of formula (1):

```
NH-      10                  30                  50
EFTFYDENDQ PINFDNALLS VASLNREHNS IEMAKDYSGT FIKISGSSIG EKNGMIYATE
         70                  90                  110
TLNFKQGQGG ARWTMYPNRQ PGSGWDSSDA PNSWYGAGAI SMSGPTNHVT VGATSATNVM
        130                 150                 170
SVAEMPQVPG RDNTEGKRPN IWYSLNGKIR AVDVPKITKE KPTPPVAPTE PQAPTYEVEK
        190                 210                 230
PLEPAPVAPS YENEPTPPVK TPDQPEPSKP EEPTYETEKP LEPAPVAPNY ENEPTPPVKT
        250                 270                 290
PDQPDPSKPE EPNYETEKPL EPAPVAPSYE NEPTPPVKTP DQPEPSKPEE PNYDPLPTPP
        310                 330                 350
LAPTPKQLPT PPAVPTVHFH YNRLFAQPQI NKEIKNEDGV DIDRTLVAKQ SVVKFELKTE
        370                 390                 410
ALTAGRPKTT SFVLVDPLPT GYQFDLEATK AASKGFETSY DKASHTVTFK ATEETLAAFN
        430                 450                 470
ADLTKSFETL YPTVVGRVLN DGATYTNNFT LTVNDATGVK SNIVRVTTPG KPNDPDNPNN
        490                 510                 530
NYIKPLKVNK NKQGVNIDGK EVLAGSTNYY ELTWDLDQYK GDKSSKEAIQ NGFYYVDDYP
        550                 570                 590
EEALTLQPEL VKIRDLEGNL VSGISVQQFD SLERAPKKVQ DLLKKANITV KGAFQLFSAD
        610
NPAEF
```

or an immunogenic fragment thereof or an analogue thereof.

2. A bacterial protein according to claim 1 further characterised by either one or both of the following features:-

(1) It is an immunodominant conserved antigen; and

(2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection;

3. A bacterial protein according to either one of claims 1 or 2 further characterised in that it is involved in binding to heart valves.

4. A bacterial protein or fragment or analogue according to any one of claims 1 to 3 wherein the protein is obtained from any one of the group of Streptococcus oralis, Streptococcus sobrinus, Streptococcus gordonii, Streptococcus sanguis, Streptococcus mutans, Streptococcus mitis, Streptococcus mitior, Streptococcus parasanguis, Streptococcus bovis, Enterococcus faecalis and Enterococcus faecium.

5. A bacterial protein or fragment or analogue according to any one of claims 1 to 3 wherein the protein is obtained from either one of the group of vancomycin-resistant Enterococcus faecalis and Enterococcus faecium.

6. A bacterial protein or fragment or analogue according to any one of claims 1 -5 wherein it is a recombinant protein, fragment or analogue.

7. A fragment or an analogue thereof according to any one of claims 1 - 6 wherein it comprises any one of the sequences of YEVEKPLEPAPVAPS, SYENEPTPPVKTPD, KTPDQPEPSKPEEPT, EPAPVAPSYENEPTP, YEVEKELVDLPVEPS, KTPDQNIPDKPVEPT, TMYPNRQPGSGWDSS and WYSLNGKIRAVDVPK.

8. A derivative of a protein, fragment or analogue according to any one of claims 1 to 7 wherein the derivative inhibits said protein.

9. An inhibitor according to claim 8 wherein it comprises isolated and purified antibody specific to the protein, fragment or analogue of any one of the preceding claims.

10. An antibody according to claim 9 wherein it is either monoclonal or polyclonal.

11. An antibody according to either one of claims 9 or 10 wherein it comprises a whole antibody or an antigen binding fragment thereof.

12. An antigen binding fragment according to claim 11 wherein it comprises any one of the group of F(ab')2, Fab', Fab and Fv fragments.

13. An antibody or antigen binding fragment according to any one of claims 9 to 12 wherein it is a human recombinant antibody or antigen binding fragment.

14. The protein, fragment, analogue, inhibitor, antibody or antigen binding fragment according to any one of the preceding claims when used in a method of diagnosis or treatment of the human or animal body.

15. The protein, fragment, analogue, inhibitor, antibody or antigen binding fragment according to claim 14 wherein the diagnostic test method is selected from one of the group of enzyme-linked immunosorbent assay, radioimmunoassay, latex agglutination assay and immunoblot assay.

16. A composition for use in a method of diagnosis or treatment of the human or animal body comprising the protein, fragment, analogue, inhibitor, antibody or antigen binding fragment of any one of claims 1 to 13 together with a pharmaceutically acceptable carrier, diluent or excipient.

17. A DNA sequence coding for a bacterial protein or an immunogenic fragment or an analogue thereof expressed during infection due to streptococci or enterococci having substantially the nucleotide sequence of formula (2):

```
5'       10                  30                  50
GAATTCACCT TCTACGATGA AAATGACCAA CCAATTAATT TTGACAATGC TCTTCTTTCA
        70                  90                 110
GTAGCCTCAC TTAACCGTGA GCATAACTCT ATTGAGATGG CTAAGGATTA TAGTGGTACT
       130                 150                 170
TTTATTAAAA TCTCAGGTTC ATCCATCGGT GAAAAAAATG GCATGATTTA TGCCACAGAA
       190                 210                 230
ACCCTGAACT TTAAACAAGG ACAGGGTGGA GCTCGCTGGA CAATGTATCC AAATCGTCAG
       250                 270                 290
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CCAGGTTCAG GTTGGGATTC ATCAGATGCA CCAAACTCTT GGTACGGTGC AGGGGCCATT

       310                   330                   350
AGTATGTCCG GTCCTACGAA TCACGTTACA GTTGGTGCAA CATCTGCTAC CAATGTGATG
       370                   390                   410
TCCGTAGCAG AAATGCCTCA AGTACCTGGA AGAGACAATA CTGAAGGTAA AAGACCAAAC
       430                   450                   470
ATCTGGTACT CACTCAATGG TAAAATTCGT GCGGTTGACG TTCCGAAAAT TACAAAAGAA
       490                   510                   530
AAACCAACTC CACCGGTAGC ACCAACTGAA CCACAAGCTC CTACCTATGA AGTGGAGAAA
       550                   570                   590
CCACTGGAAC CGGCTCCAGT AGCACCAAGC TACGAAAATG AGCCAACTCC ACCAGTAAAA
       610                   630                   650
ACTCCAGATC AACCGGAGCC ATCAAAACCA GAAGAGCCAA CATATGAGAC AGAGAAACCA
       670                   690                   710
TTGGAACCAG CTCCAGTAGC ACCAAACTAC GAAAATGAGC CAACTCCACC AGTAAAAACT
       730                   750                   770
CCAGATCAAC CAGACCCATC AAAACCGGAA GAGCCAAACT ATGAGACAGA GAAACCATTG
       790                   810                   830
GAACCAGCTC CAGTAGCACC AAGCTATGAA AATGAGCCAA CTCCACCGGT AAAAACTCCA
       850                   870                   890
GATCAACCAG AGCCATCAAA ACCAGAAGAG CCAAATTATG ATCCATTGCC AACTCCGCCG
       910                   930                   950
CTAGCACCAA CTCCTAAGCA GTTGCCAACA CCACCAGCGG TGCCAACAGT TCACTTCCAT
       970                   990                  1010
TACAATCGTC TATTTGCACA ACCTCAGATT AATAAAGAAA TTAAAAACGA GGATGGAGTA
      1030                  1050                  1070
GATATTGATC GTACTCTAGT TGCTAAGCAG TCTGTAGTGA AGTTTGAGCT GAAAACAGAA
      1090                  1110                  1130
GCTTTAACTG CTGGTCGTCC AAAAACAACT TCGTTTGTAT TGGTAGATCC ACTTCCAACT
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      1150                1170                1190
GGCTATCAGT TTGATTTGGA AGCAACCAAG GCTGCAAGCA AAGGTTTTGA AACAAGCTAT
      1210                1230                1250
GACAAAGCTA GTCACACTGT AACCTTTAAG GCTACTGAGG AGACCTTAGC TGCTTTCAAT
      1270                1290                1310
GCTGATTTGA CAAAATCCTT TGAGACTCTA TATCCAACTG TTGTTGGTCG TGTCTTGAAT
      1330                1350                1370
GATGGGGCGA CTTATACGAA TAACTTTACA TTGACAGTCA ACGATGCTAC TGGTGTCAAG
      1390                1410                1430
TCAAACATTG TTCGTGTAAC GACTCCAGGT AAACCAAATG ATCCTGACAA TCCAAATAAC
      1450                1470                1490
AACTACATCA AGCCTTTGAA AGTTAACAAG AACAAGCAAG GTGTGAATAT TGATGGCAAA
      1510                1530                1550
GAAGTTCTAG CTGGTTCAAC GAACTACTAT GAACTCACAT GGGATTTGGA TCAATACAAG
      1570                1590                1610
GGAGATAAAT CTTCTAAAGA AGCGATTCAA AATGGTTTCT ACTATGTGGA TGATTATCCA
      1630                1650                1670
GAAGAAGCTT TAACGCTTCA ACCAGAATTG GTTAAGATTC GTGATCTAGA GGGCAACCTT
      1690                1710                1730
GTATCAGGTA TCAGTGTTCA ACAGTTTGAT AGTTTAGAAC GTGCGCCTAA GAAGGTTCAA
      1750                1770                1790
GATCTGTTGA AGAAAGCAAA CATCACTGTT AAAGGTGCTT TCCAACTCTT CTCAGCTGAT
      1810
AATCCAGCTG AATTC
```

and homologues thereof.

18. A DNA sequence according to claim 17, the protein for which it encodes being further characterised by either one or both of the following features:-

(1) It is an immunodominant conserved antigen; and

(2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection; and

19. A DNA sequence according to either one of claims 17 or 18 further characterised in that the protein for which it encodes is involved in binding to heart valves.

20. An expression vector including substantially the DNA sequence of any one of claims 17 to 19.

21. A DNA probe specific to the DNA sequence of any one of claims 17 to 19.

22. A derivative of a DNA sequence according to any one of claims 17 to 19 wherein it inhibits the synthesis or activity of the protein.

23. The DNA sequence, vector, probe or inhibitor according to any one of claims 17 to 22 when used in a method of treatment or diagnosis of the human or animal body.

24. Fibronectin or an immunogenic fragment thereof or an analogue thereof or an antibody thereto or an antigen binding fragment thereof when used in a method of treatment or diagnosis of the human or animal body for infection due to Streptococci or Enterococci.

25. Fibronectin or an immunogenic fragment thereof according to claim 24 wherein the fibronectin is human fibronectin.

26. Antibodies specific to HSP 90 or immunogenic fragments or analogues thereof for use in a method of diagnosis or treatment of the human or animal body of infection due to streptococci or enterococci due to any one of the group of S.oralis, S.gordonii and S.sanguis.

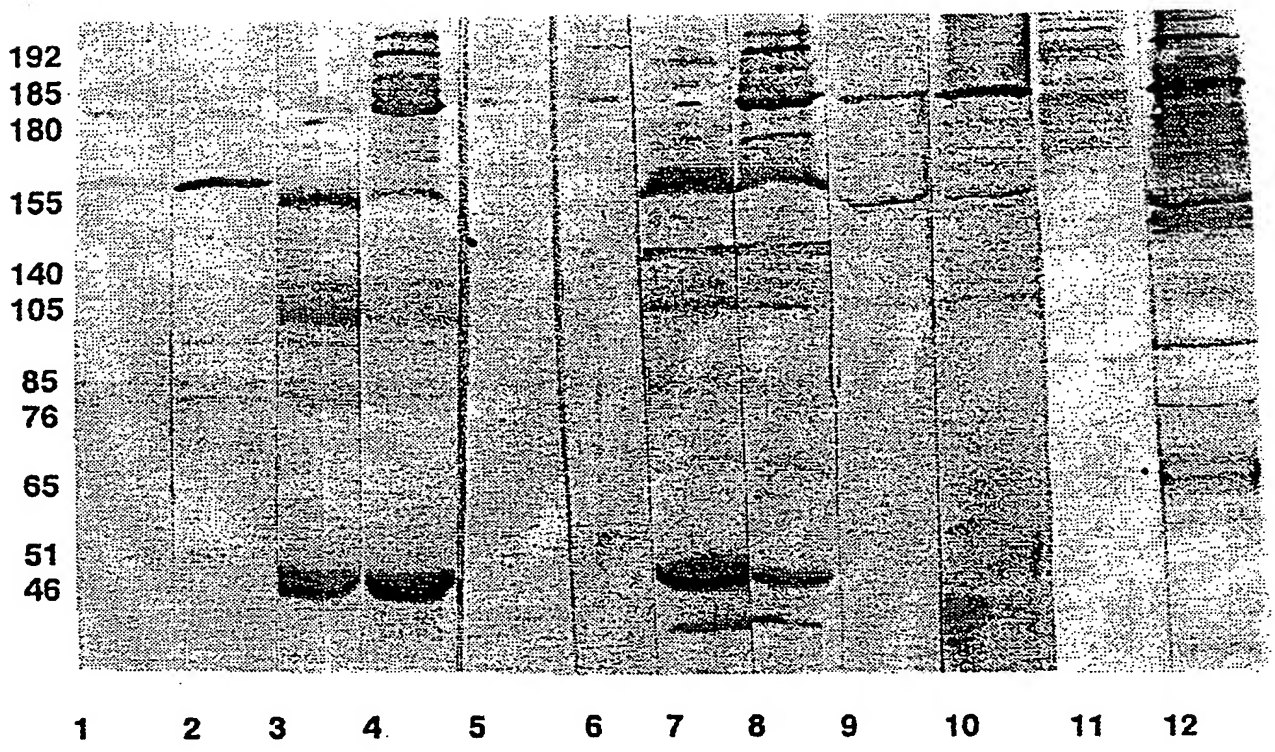

FIGURE 1

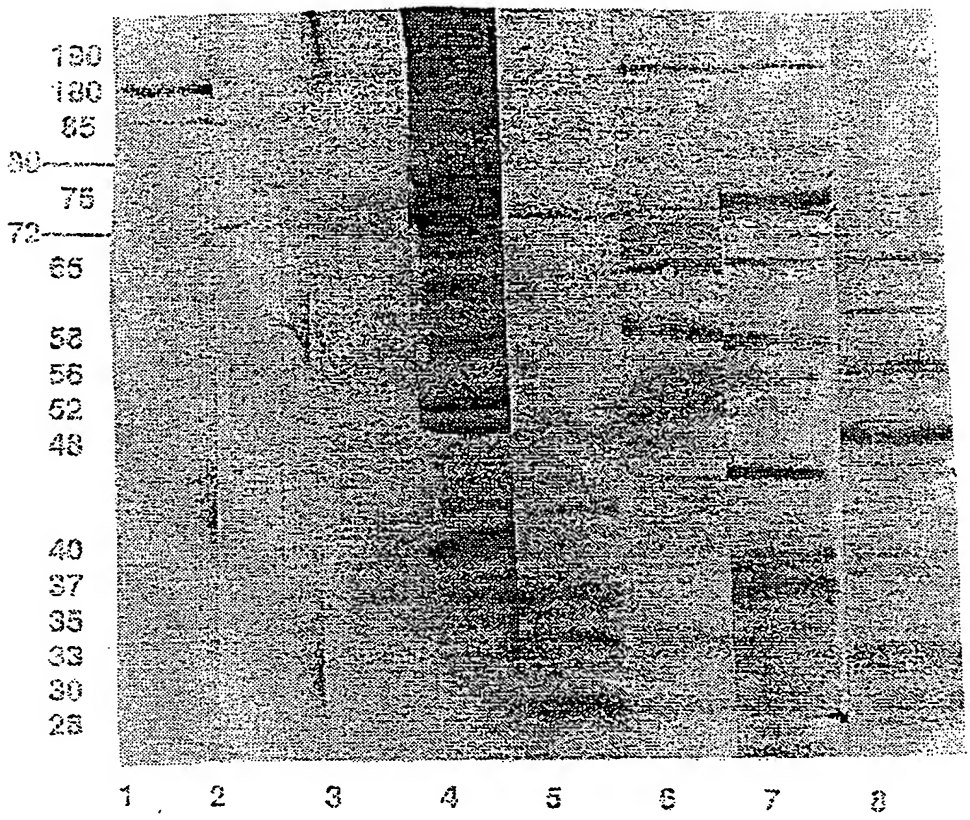

FIGURE 2

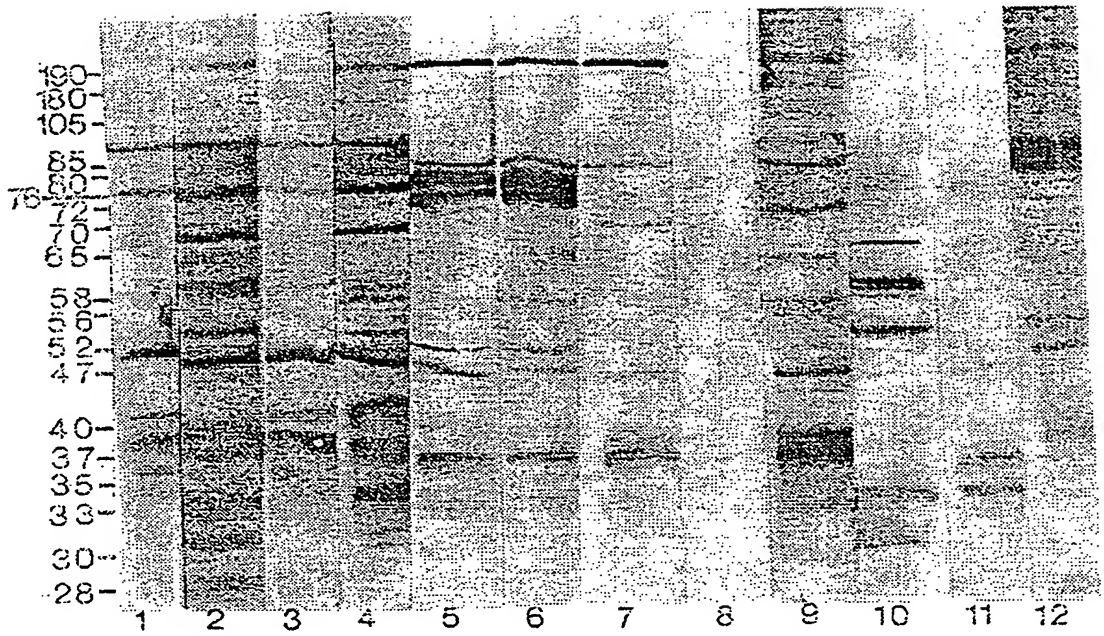

Figure 3

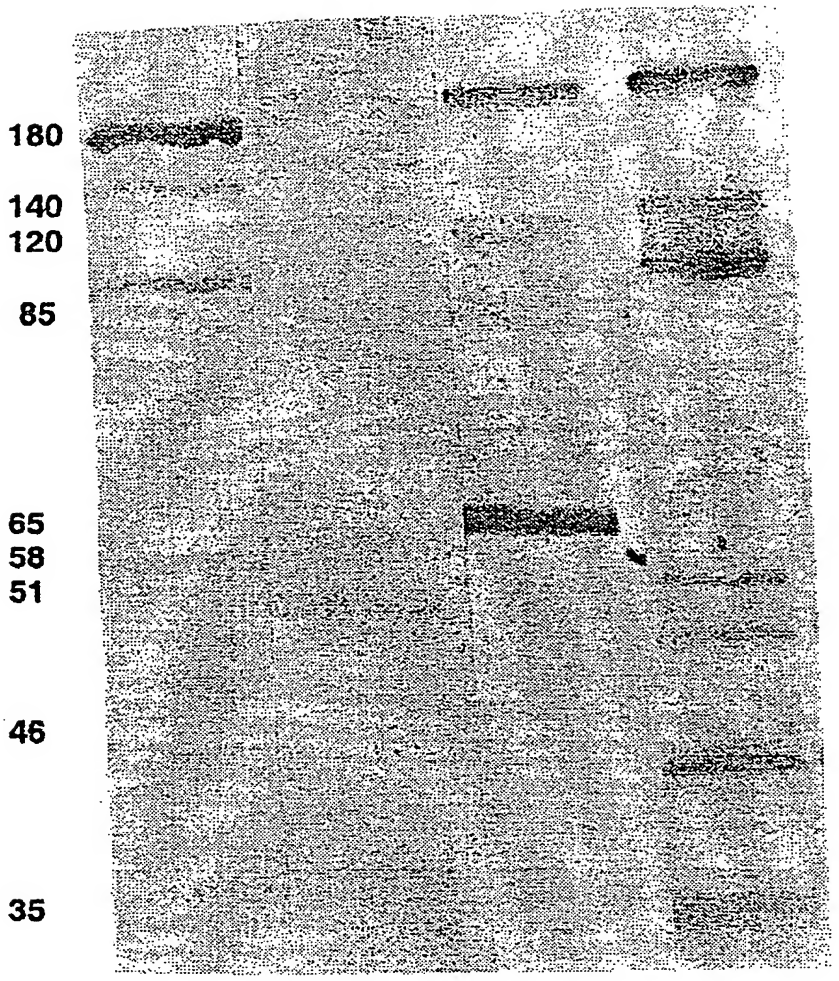

FIGURE 4

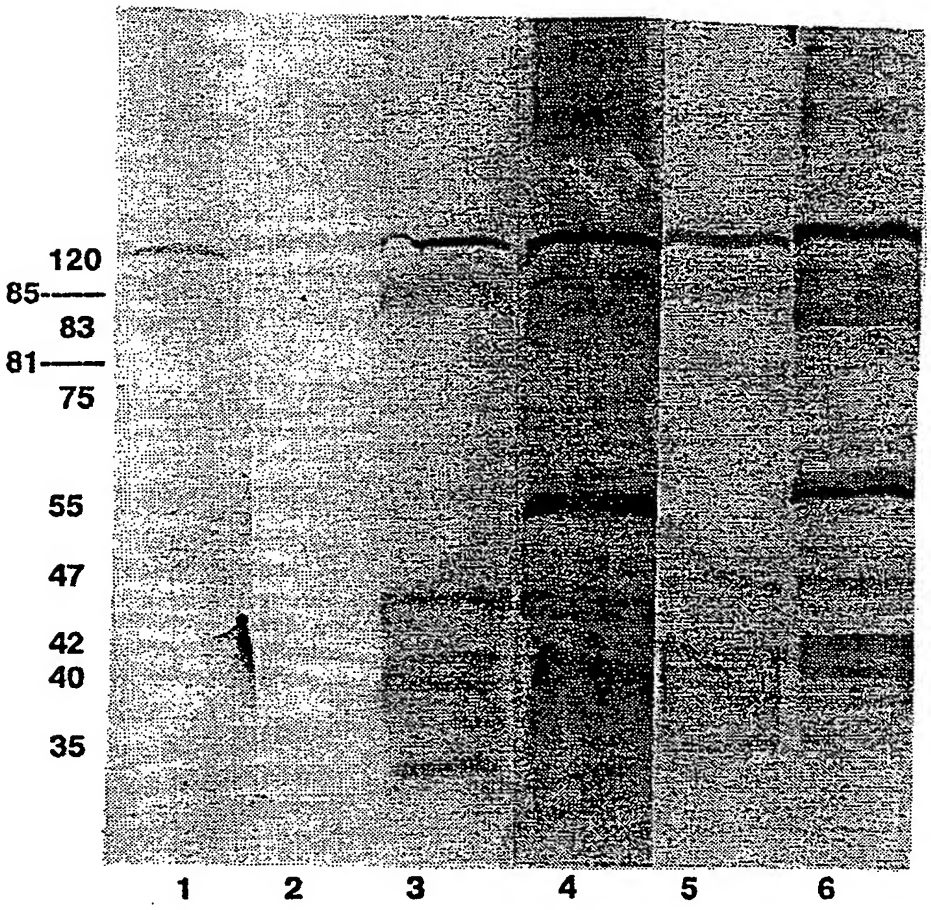

FIGURE 5

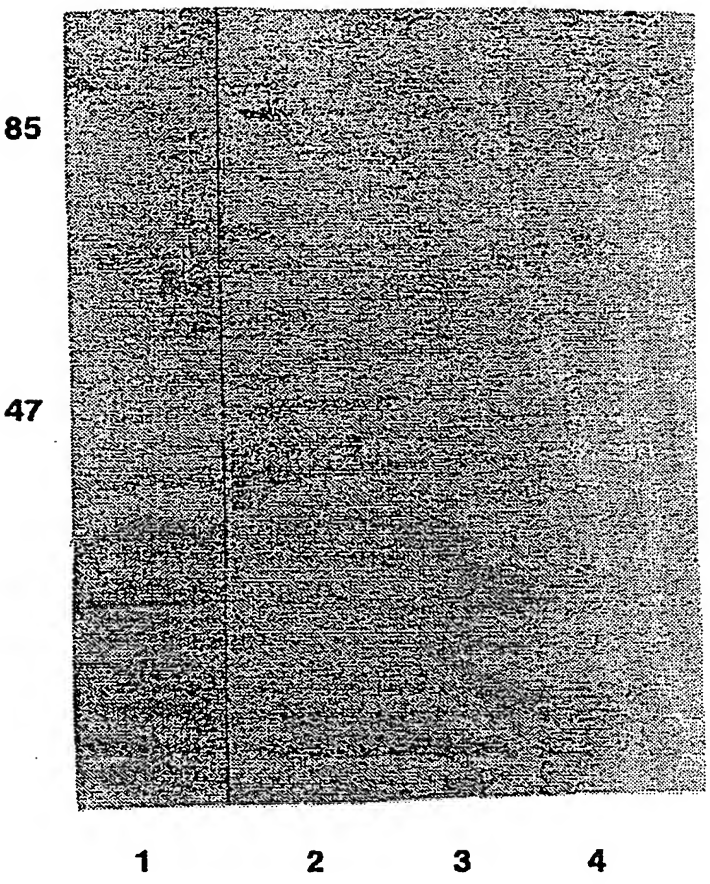

FIGURE 6

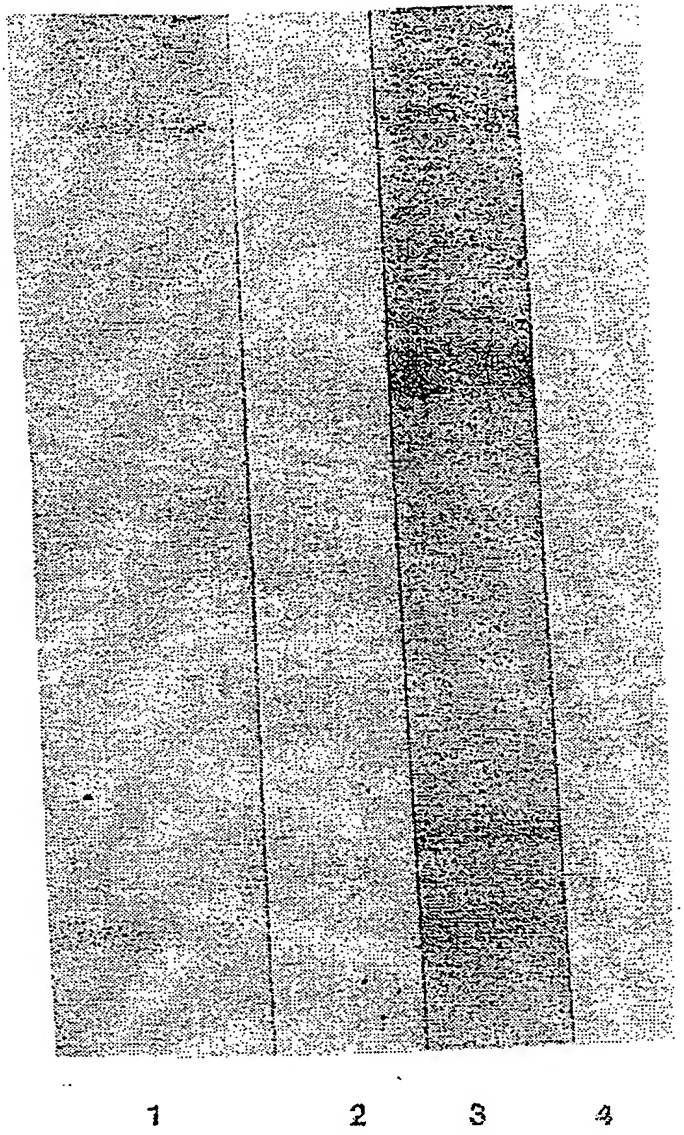

FIGURE 7

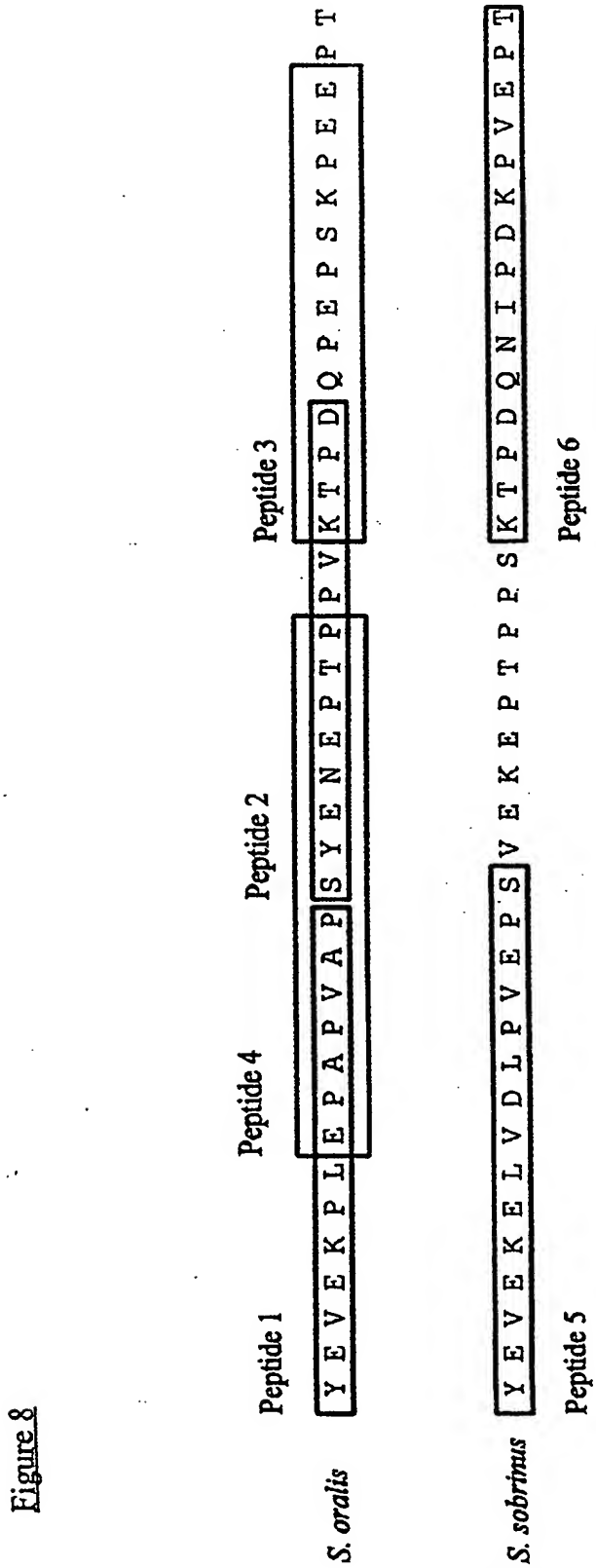
Peptide 1
Peptide 4
Peptide 2
Peptide 3
Peptide 5
Peptide 6
S. oralis
S. sobrinus
Y E V E K P L E P A P V A P S Y E N E P T P P V K T P D Q P E P S K P E E P T
Y E V E K E L V D L P V E P S V E K E P T P P S K T P D Q N I P D K P V E P T

Figure 8

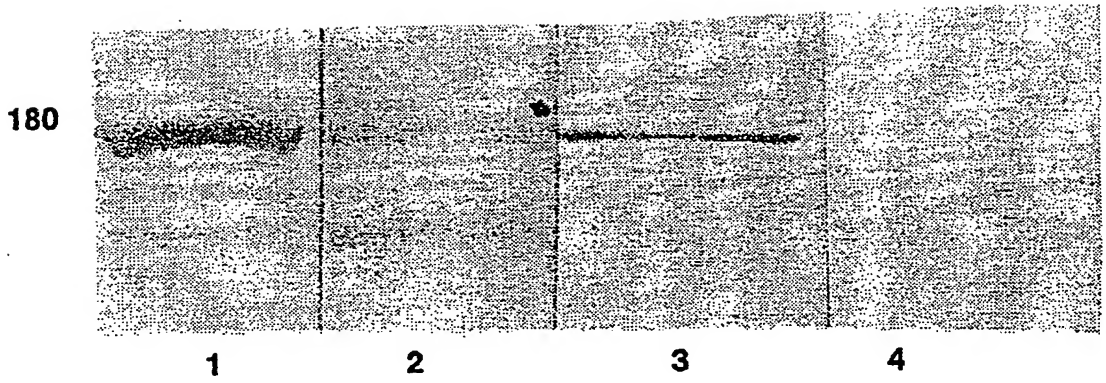

FIGURE 9

Figure 10

Gap alignment of the *S. sobrinus* antigenic region

with the human Fibronectin primary sequence

```
                    710                 730                 750
FN HUM      STSTPVTSNT VTGETIPFSP LVATSESVTE ITASSFVVSW VSASDTVSGF

ESA STRSO   .......... .......... .......... .......... .........Y
                    760                 770                 790
FN HUM      RVEYELSEEG DEPQYLDLPS TATSVNIPDL LPGRKYIVNV YQISEDGEQS

ESA STRSO   EVEKELVDLP VEPSYEKEPT PPS....... .......... ..........
                    810                 830                 850
FN HUM      LILSTSQTTA PDAPPDPTVD QVDDTSIVVR WSRPQATITG YRIVYSPSVE

ESA STRSO   ...KTPDQNI PDKPVEPT.. .......... .......... .........
```

Key for Figure 10:

FN HUM — the human fibronectin primary sequence homologous to the ESA STRSO peptin region.

ESA STRSO — the proposed S.sobrinus endocarditis specific antigenic site.

The numbers correspond to the amino acids of the complete human fibronectin primary sequence.

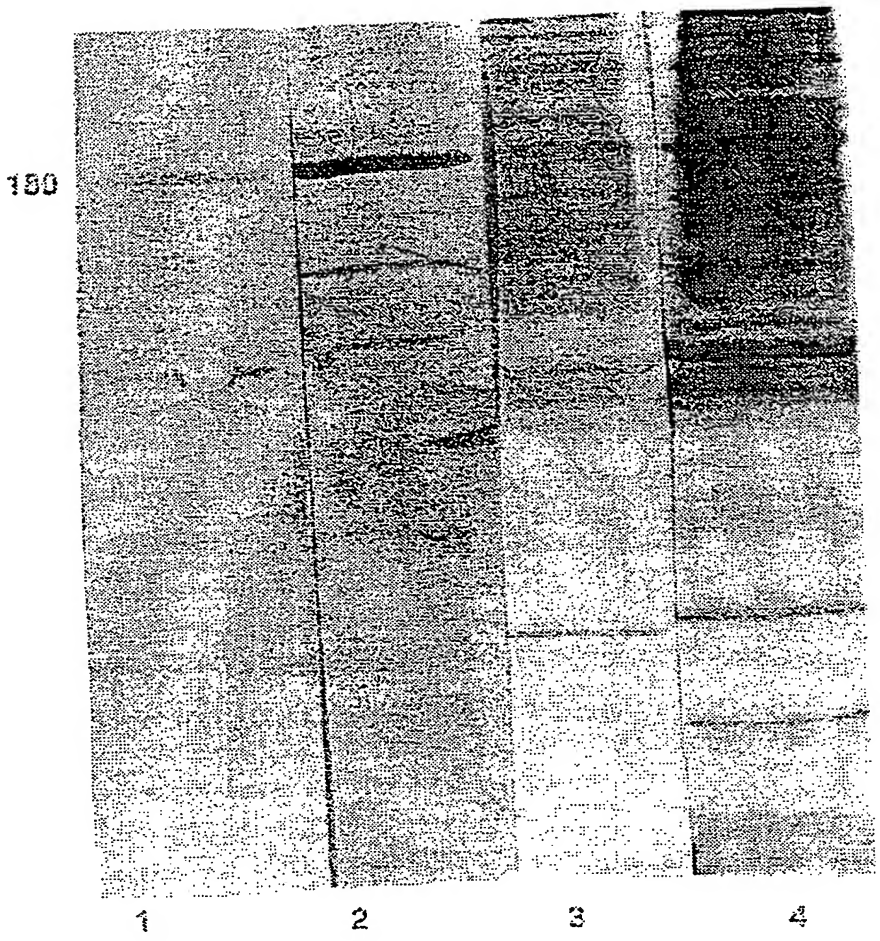

FIGURE 11